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STUDIES ON THE VIRUS OF NOTHOSCORDUM MOSAIC

H. H. McKinney[1]

(Accepted for publication December 14, 1949)

Mosaic on *Nothoscordum fragrans* (Vent.) Kunth, false garlic, was reported and illustrated by the writer in 1929,[2] in connection with a general description of some of the mosaics observed in the Canary Islands. Mosaic plants have been maintained in culture from the bulbs since 1927. As the host is of little importance, the virus has been studied as time permitted.

In 1945, mosaic-diseased *Nothoscordum fragrans* collected at Baton Rouge, Louisiana, by Douglas C. Bain[3] was sent to Dr. Philip Brierley, who transmitted the material to the writer for comparison with the collection mentioned above.

MATERIALS AND METHODS

The virus was obtained in juice from leaves taken from plants having the mosaic disease. The host plant was maintained in a greenhouse without difficulty. Propagation was more successful by bulbs than by bulblets, since a rather high percentage of the latter failed to grow. Also, a considerable number of the bulblets that did grow failed to carry the virus, whereas the bulbs were uniformly infected. The virus is not transmitted through the seed, and healthy plant stock was obtained from seedlings. The seeds were sown about 1/16 in. deep in a pot of fine soil or sand which was covered with porous paper. The paper and the soil or sand were kept moist at all times. The paper was removed when emergence started. Temperatures of 16° to 20° C. favored seed germination. When the seedlings were 2 to 3 in. tall they were transplanted to soil in 3-in. clay pots, one seedling in each pot.

The plants were maintained at temperatures ranging from 16° to 24° C., before and after inoculation, but stock virus was retained in the host during the summer months at the prevailing temperatures, and the mosaic symptoms persisted during this period when the plants were kept watered. Seedlings were inoculated when they were 3–5 in. tall, by wiping all of the leaves with a virus preparation to which No. 600 carborundum powder had been added. Symptoms appeared in 11–24 days after inoculation.

HOST RANGE

Nothoscordum fragrans is the only species tested thus far that has shown signs of infection. Fresh undiluted extracts have rarely failed to infect all

[1] The author is indebted to Mathew Koerner for assisting in the conduct of these studies.

[2] McKinney, H. H. Mosaic diseases in the Canary Islands, West Africa, and Gibraltar. Jour. Agr. Res. [U.S.] 39: 557–578. 1929.

[3] See U. S. Dept. Agr., Bur. Plant Indus., Soils, and Agr. Engin., Plant Dis. Rptr. 29: 389. 1945.

of the inoculated seedlings in a test, and usually all seedlings become infected from extracts diluted 10^{-1}, and sometimes from dilutions of 10^{-2}. Resistance increases with the age of the seedlings.

The species listed below have been inoculated with fresh juice from mosaic leaves, and the plants were cultured under conditions that favor infection by other viruses to which they are susceptible. All plants failed to show signs of disease. All virus preparations used were known to be active as they induced mosaic on control plants of *Nothoscordum fragrans*.

Allium cepa L.	Onion var. Ebenezer
A. vineale L.	Wild garlic, field garlic
Lilium formosanum Stapf	Lily
Ornithogalum thyrsoides Jacq.	Ornithogalum
Sparaxis tricolor Ker	Wandflower
Avena sativa L.	Oats var. Letoria
Triticum aestivum L.	Wheat var. Michigan Amber
Zea mays L.	Corn var. Golden Giant Sweet
Datura stramonium L.	Jimsonweed
Lycopersicon esculentum Mill.	Tomato var. Marglobe
Nicotiana tabacum L.	Tobacco var. Samsun
Beta vulgaris L.	Sugar beet
Cucumis sativus L.	Cucumber var. Early White Spine
Phaseolus vulgaris L.	Bean var. Scotia
Sesamum indicum L.	Sesame

PROPERTIES OF THE VIRUS

Dilution. The infection end point for the virus in fresh plant juice, when diluted in distilled water, usually extends slightly beyond 10^{-4}, and sometimes slightly beyond 10^{-5}.

Thermal death point. In 5-ml. samples of fresh plant juice, contained in 50-ml. Erlenmeyer flasks, held for 10 min. in a water bath, the virus became inactive at 58° to 60° C.

Longevity when stored. When virus was stored in plant juice at room temperature, 33.3 per cent of the test plants became infected from juice stored for 5 days and 20 per cent from juice stored for 17 days; no infection occurred after 20 days' storage. When stored at 4° to 5° C., 23 per cent of the test plants became infected from juice stored for 52 days.

When the virus was stored in fresh leaves in tightly stoppered tubes at 4°–5° C., 15.4 per cent of the test plants became infected after storage for 177 days. When mosaic leaves were dried and stored at the laboratory temperatures, 100 per cent of the test plants became infected after storage for 5 days, 40 per cent became infected from tissue stored for 40 days, and no infection occurred after 43 days' storage.

Filtration. Fresh juice from infected leaves was heated for 10 min. at 42.5°–45° C., then centrifuged for 2 min. in a 3-in. cup of a super-centrifuge. The clarified fluid was then filtered at room temperature. The virus passed new Berkefeld "V" and "N" filters, but it did not pass a new Berkefeld "W" filter, a Seitz asbestos-pad filter, or a Schott "G5 on 3" filter having an average pore size of 0.78 μ.

The virus that was collected in Louisiana was not studied so extensively

as the one collected on Teneriffe, but the two collections seemed to be alike in all comparisons.

The present studies have failed to reveal the identity of the virus of Nothoscordum mosaic as that of any previously described virus that induces mosaic in members of the Gramineae or the Liliaceae. Accordingly, the virus is here named and described.

TECHNICAL DESCRIPTION OF THE VIRUS

Marmor angustum sp. nov.

Specific name from Latin *angustus*, adj., confined within narrow limits, referring to the apparently narrow host range.

Common name: Nothoscordum-mosaic virus.

Host reactions: in *Nothoscordum fragrans*, no local lesions; induces light green chlorosis which is studded with normal green patches, producing a typical mosaic mottling in the leaves. Symptoms persist over a wide range of conditions, with no signs of evanescence. Thus far, infection has been confined to *N. fragrans*. Nothoscordum mosaic is illustrated in another paper,[2] figure 1, page 558.

Transmission: By inoculation with expressed juice from mosaic-diseased leaves, readily by wiping the leaves of small healthy seedlings in the presence of carborundum powder (No. 600); difficult in older plants; cool growing temperatures (16°–20° C.) most favorable for infection; incubation period 11 to 24 days. The virus is transmitted through the bulbs and a portion of the bulblets, but not through the seed.

Physical properties: The dilution end point of the virus in water is between 10^{-4} and 10^{-5}. Inactivated in 10 min. at temperatures near 58°–60° C. in fresh plant juice. Activity very low or null in plant juice after storage for about 20 days at room temperature, and low after storage for 52 days at 4°–5° C.; low after 177 days' storage of fresh mosaic leaves at 4°–5° C.; very low or null after 43 days in air-dry mosaic-leaf tissue kept at room temperature. Virus passed new Berkefeld "V" and "N" filters, but not a new Berkefeld "W," the Seitz asbestos-pad filter, nor the Schott "G5 on 3" filter with average pore size 0.78 μ.

Distribution: Teneriffe, Canary Islands, and Louisiana.

SUMMARY

A light green mosaic mottling in *Nothoscordum fragrans* (false garlic) is caused by a filterable virus that is readily transmissible by wiping the leaves of young healthy seedlings with the juice from the diseased leaves in the presence of carborundum powder.

Of the 16 species from 15 genera that were tested, only *Nothoscordum fragrans* was found to be susceptible.

The dilution end point of the virus in water was found to be between 10^{-4} and 10^{-5}, and the thermal death point is between 58° and 60° C. in 10 min. Infectivity was lost when the juice from infected leaves was stored

at room temperature for 20 days, and it was reduced when the juice was stored at 4°–5° C. for 52 days. When the mosaic-diseased leaf tissue was kept air dry in the laboratory for 40 to 43 days, the activity of the virus was low or null. The virus passed the Berkefeld "N" filter, but not the Berkefeld "W" nor finer filters.

The virus is designated *Marmor angustum* sp. nov.

PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

THE RECOVERY OF WESTERN X-DISEASE OF PEACH FROM MONTMORENCY CHERRY AND ITS RELATION TO BUCKSKIN OF SWEET CHERRY[1]

S. M. Zeller[2] and J. A. Milbrath

(Accepted for publication December 22, 1949)

Little-cherry has been found in Montmorency cherry trees in Wasco County, Oregon, for a number of years. Trees with the symptoms were first observed in 1942, but some sour-cherry orchards had been removed prior to that time, reportedly because of this disease. The disease is of considerable economic importance in Oregon. Preliminary studies of this disease have been reported by Zeller and Milbrath (7).

SYMPTOMS

The symptoms usually start in a single branch, or even a twig, although at times they have been observed in more than one twig on the same tree. Within a year or two several large branches or the entire tree is affected.

The disease spreads rapidly through an orchard. In July 1947 a block of Montmorency was observed in which it was difficult to find a tree without some infection. Just two years previously this orchard was inspected and there was no indication of infection.

For the most part the one outstanding symptom is the reduced size of fruits, with an attendant delay in ripening. Fruits of Montmorency cherry remain small, roundish, and green even when normal fruits are large and becoming red (Fig. 1). The fruits gradually change to light green or yellow and become soft. There is not the tendency to become conical at maturity as do sweet cherries with the buckskin disease (Fig. 2). As the abnormal fruits ripen they have a puttylike consistency and a livid to pinkish color, usually with a dull surface. Pockets of internal necrotic tissue can be observed beneath the skin. Some of the fruit dries on the tree and may remain attached until the next season.

Whereas sweet-cherry trees in this area do not deteriorate upon infection with this disease, Montmorency trees begin a slow decline by the time a large portion or the whole tree shows infection. At first such trees are off-color, with a light greenness in the leaves, and have a scarcity of leaves on certain branches or twigs and a general appearance of slow decline. Individual leaves do not have definite symptoms at first, but soon there is a tendency for leaf side-margins to roll upward lengthwise, and a bronzing often appears. It is not known whether this is a specific leaf symptom on

[1] Published as Technical Paper No. 595, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

[2] Deceased November 4, 1948.

buckskin-infected Montmorency trees, or a general and usual symptom with Montmorency trees in decline. Dieback of twigs, or even larger branches, in infected trees is not unusual.

Naturally infected Montmorency trees with the small fruits have been observed to behave about the same whether on Mazzard or Mahaleb roots.

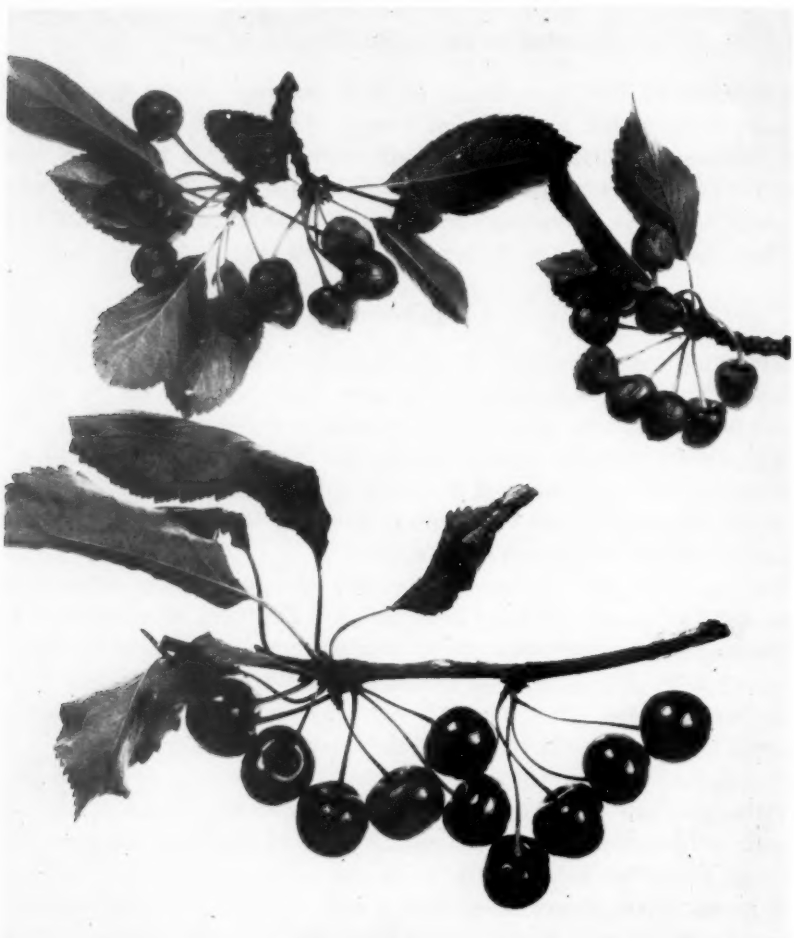

FIG. 1. Buckskin of Montmorency cherry. Upper, spurs and fruit from diseased tree. Lower, twig from healthy tree.

NATURE OF THE DISEASE AND RELATION TO OTHER DESCRIBED DISEASES

This disease in sour cherries has been transmitted by the budding and grafting methods usually employed with stone-fruit viruses, which indicates that it is a virus disease. In nature the transfer of the virus is doubtless brought about by an insect vector, although the vector has not been determined.

The disease has been transmitted by budding from diseased Montmorency, not only to healthy Montmorency cherry trees but to sweet cherry (Black Republican variety) in which it appears as the buckskin disease, and to healthy J. H. Hale and Lovell seedling peach trees where it results in western X-disease.

There is one serious difficulty which usually arises when an attempt is made to transmit a disease like buckskin of cherry (sweet or sour) to a peach tree. The cherry inoculum usually carries besides the virus in question one or more latent viruses, such as ring spot (1), to which peach trees

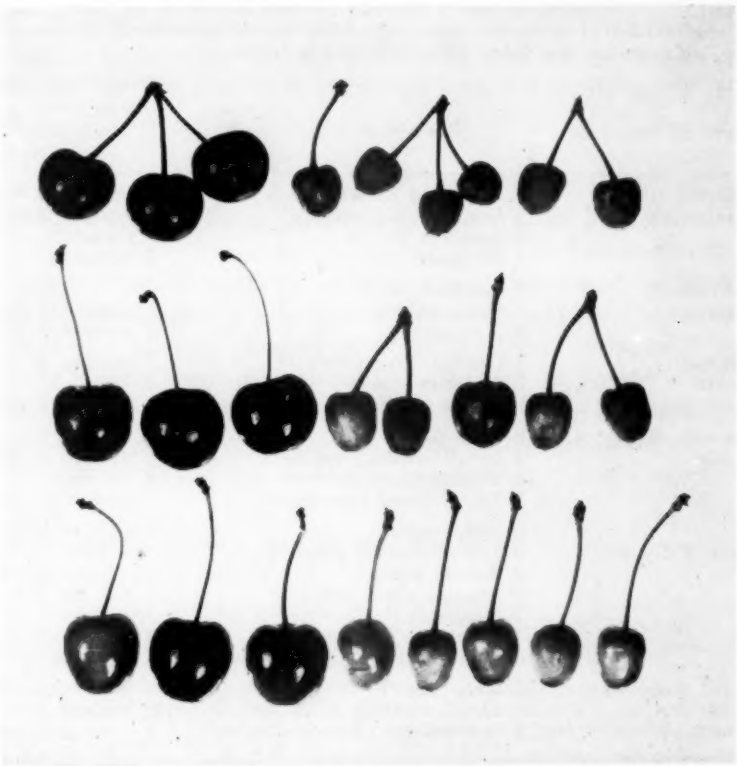

FIG. 2. Fruit symptoms of buckskin in Montmorency, Bing, and Napoleon cherries. The three cherries on the left in each row are normal.

react more or less violently. Apparently in such cases the bud is often killed by virus contamination before the virus being studied has had a chance to move out of the bud and infect the test tree. Then, too, it is generally difficult to transmit virus to a peach tree in poor vigor. A much higher percentage of transfers can be expected if the same peach tree is inoculated two years in succession from the same sweet- or sour-cherry source tree. The first year the peach tree may only be conditioned to the ring spot or latent virus. The second year there will be no reaction to the latent virus and the bud may callus in and better organic union may

result. By this method a fairly good percentage of "take" was obtained in inoculating peach trees with the virus from sour cherry, when a latent-virus complex was present. It was thus possible also to inoculate peach trees with little-cherry from sweet cherries in Wasco county, Oregon, and produce western X-disease in the inoculated peach trees. In 14 such cases results were positive (Table 1). The table also shows positive results of inoculations from 1) sweet cherry with buckskin disease to healthy sweet cherry, producing buckskin disease; 2) peach with western X-disease to healthy chokecherry, producing red-leafed chokecherry; 3) red-leafed choke-

TABLE 1.—*Inoculations which indicate western X-disease of peach, buckskin of cherry, and red-leafed chokecherry are all caused by the same virus, in Wasco county, Oregon. All inoculum was taken from trees in The Dalles area*

Source of inoculum	Number of inoculations	Number of positive infections and symptoms
Bing cherry, buckskin	7 Improved Elberta peach	7 western X-disease
	1 Oregon Cling peach	1 western X-disease
	8 Lambert cherry	4 buckskin
	1 Napoleon cherry	1 buckskin
Napoleon cherry, buckskin	2 Lambert cherry	0
	1 Orange Cling peach	1 western X-disease
	4 Hale peach	1 western X-disease
Lambert cherry, buckskin	1 Orange Cling peach	1 western X-disease
	2 Lambert cherry	0
	2 Lovell seedling peach	1 western X-disease
Montmorency cherry, buckskin	2 Hale peach	2 western X- disease
	3 Lovell seedling peach	2 western X- disease
	6 Montmorency cherry	4 buckskin
	3 Black Republican cherry	1 buckskin
Peach, western X-disease	5 Hale peach	3 western X-disease
	5 Late Crawford peach	1 western X-disease
	5 Salway peach	1 western X-disease
	5 Phillips Cling peach	1 western X-disease
	7 Rochester peach	4 western X-disease
	21 Lovell seedling peach	7 western X-disease
	10 Chokecherry (*Prunus demissa*)	4 red-leafed chokecherry
Red-leafed chokecherry (*Prunus demissa*)	5 Hale peach	2 western X- disease
	10 Lovell seedling peach	2 western X- disease
	11 Chokecherry (*Prunus demissa*)	4 red-leafed chokecherry

cherry to healthy peach, producing western X-disease; 4) red-leafed chokecherry to healthy chokecherry, producing red-leafed chokecherry; and from 5) peach with western X-disease to healthy peach, producing western X-disease.

The table also indicates the varieties of cherries and peaches in each case. It is readily seen from the results of these cross inoculations that little-cherry of sweet and sour cherries in Wasco county, Oregon, contains the same virus as that which causes the western X-disease of peach in the same region, and that the red-leafed condition in chokecherry in that area is also caused by the same virus.

This relationship of the buckskin disease of sweet cherry to the western

X-disease (under the name of leaf-casting yellows) of peach was first demonstrated by Rawlins and Thomas (5) in California. We have been unable to see any difference between the symptoms of the buckskin disease in Green Valley, California, and those of the buckskin disease of sweet cherry in Wasco county, Oregon, but the same relation of rootstocks to the lethal powers of the virus (4) has not been demonstrated.

Palmiter and Parker (2, 3) have associated X-disease of peach with a disease of Montmorency and English Morello in New York in which the conspicuous symptom is a little-cherry condition. Richards, Wadley, and Cochran (6) have found that the Utah X-disease complex causes a wilt and decline of sweet and sour cherry on Mahaleb root and little cherry on Mazzard root. These results with related strains of X-disease substantiate the Oregon findings (7), that the virus responsible for X-disease in peach can also cause little-cherry in sweet and sour cherries.

SUMMARY

A little-cherry disease of Montmorency cherry has been described from natural infections. Transmission of the virus to peach and to Montmorency and Black Republican cherries was demonstrated. This buckskin disease, or "little-cherry" condition, was transmitted from sweet cherry to to sweet cherry and also to peach in which it appears as western X-disease. Red-leafed chokecherry disease was transmitted to chokecherry and to peach, producing western X-disease in the latter. This indicates that western X-disease of peach, red-leafed chokecherry, and buckskin of sweet and sour cherries as they occur in Wasco county, Oregon, are caused by the same virus.

OREGON AGRICULTURAL EXPERIMENT STATION
CORVALLIS, OREGON

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RHIZOME ROT OF WHITE CALLA CAUSED BY PHYTOPHTHORA ERYTHROSEPTICA

C. M. Tompkins and C. M. Tucker

(Accepted for publication January 28, 1950)

The white calla (*Zantedeschia aethiopica* Spreng.) is grown on an extensive scale in San Francisco and San Mateo counties of California for cut-flower and rhizome production. In early August, 1946, coinciding with the end of the rhizome-harvesting period, severe rot was observed on two lots of freshly cured rhizomes, one of which had been grown in San Francisco and the other in Colma. In press at that time was a paper[1] on a leaf blight of pink calla (*Z. rehmannii* Engler), a disease associated with the same causal organism as that affecting the rhizomes of white calla. Data on the latter disease are given herewith.

SYMPTOMS OF THE DISEASE

External symptoms of the disease on the rhizomes consist of small to large lesions, irregular in shape, dark brown and water-soaked. With age these lesions become dry, shriveled, and slightly sunken. When the rhizomes are cut in cross or longitudinal sections, the internal diseased tissues appear light gray and firm but rubbery in texture, separated from healthy tissues by a definite line of demarcation (Fig. 1). The decay is odorless. Internal lesions may involve small to large areas of tissue and are in marked contrast to healthy tissue (Fig. 1, left). There may be two or more infection sites in each rhizome.

THE CAUSAL ORGANISM, PHYTOPHTHORA ERYTHROSEPTICA

Isolations made from diseased white calla rhizomes on malt-extract agar have consistently yielded a fungus which has been identified as *Phytophthora erythroseptica* Pethyb.

Studies made on an isolate of the fungus from white calla showed it to be indistinguishable, in culture, from isolates from the pink calla, and from isolates from potato tubers from Idaho and Nebraska. Comparisons of isolates from the four sources revealed similar temperature-growth relations, as well as similar growth and development of reproductive organs on various culture media.

Measurements of sporangia, oogonia, and oospores of the isolates did not provide a basis for their differentiation. For example, sporangia of the white calla isolate had mean measurements of $35.1 \times 20\ \mu$, varying less than $\frac{1}{2}\ \mu$ from those of the pink calla isolates. The oogonia, oospores, and antheridia were likewise very similar in size and appearance to those of the pink calla isolates. In no case did mean measurements of oogonia and oospores vary more than $1\ \mu$ between isolates.

[1] Tompkins, C. M., and C. M. Tucker. Leaf blight of pink calla caused by *Phytophthora erythroseptica*. Phytopath. **37**: 382–389. 1947.

Isolates from the white calla, like those from the pink calla, failed to invade and cause rotting of potato tubers, but all isolates from potatoes caused the typical pink rot frequently described as characteristic for the species. In spite of distinct differences in pathogenicity, the similarity of the calla isolates to the potato isolates in morphologic and physiologic characters is regarded as sufficient evidence to warrant their identification as *Phytophthora erythroseptica*.

An isolate of the fungus proved pathogenic to healthy white calla rhizomes secured from another source in the same vicinity. Inoculum was prepared on poured plates of malt-extract agar. Rhizomes were carefully

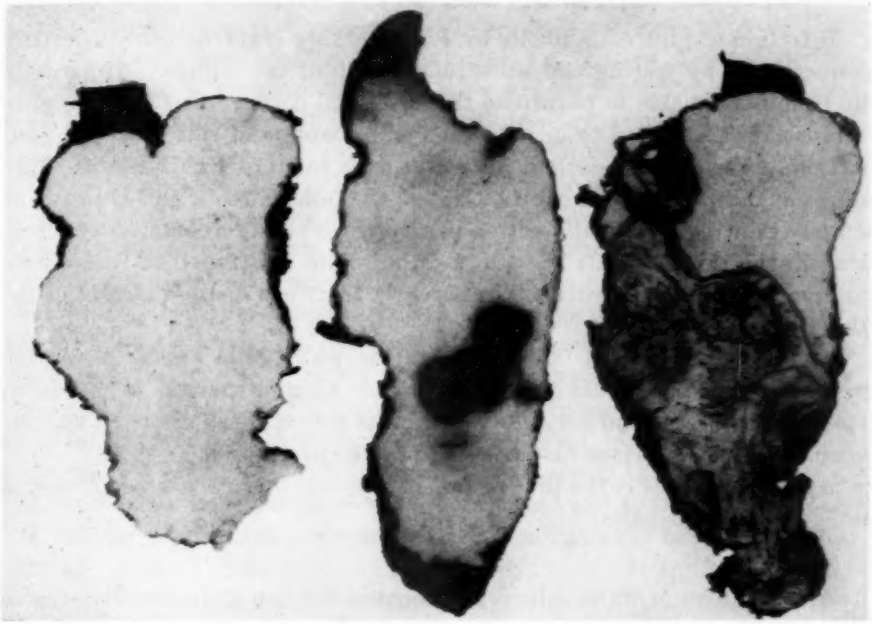

FIG. 1. Symptoms of rhizome rot of white calla (*Zantedeschia aethiopica*), caused by *Phytophthora erythroseptica* (healthy rhizome at left). In longitudinal section the tissues are firm but rubbery, light gray in color, and sharply delimited from adjoining healthy tissue.

scrubbed in water, rinsed in sterile distilled water, dried, and then placed in a moist chamber lined with wet filter paper. Small blocks of inoculum, cut with a flamed scalpel, were placed on the surface of each of 12 rhizomes and covered with moist absorbent cotton. The controls consisted of six rhizomes treated in a similar manner but using sterile malt-agar blocks instead of the inoculum. All moist chambers were then covered and removed to a cool greenhouse in which the air temperature ranged from 13° to 19° C. At the end of 10 days, nine of the inoculated rhizomes had become infected, while the controls remained healthy. The fungus was reisolated from each infected rhizome and proved to be identical with the original isolate. The reisolates proved highly pathogenic. In these tests, the infected rhizomes had symptoms identical with those of naturally infected rhizomes.

In limited infection experiments in the greenhouse, 16 pots of autoclaved soil were planted with two small white calla rhizomes per pot. Meanwhile, inoculum was prepared by growing the fungus on sterilized cracked wheat in 8-in. test tubes, and when ready for use it was added to the soil in each of 10 pots in such a manner as to avoid injuring the root system of the young plants. Sterile cracked wheat was added to the pots serving as controls. All pots were heavily watered each day so as to keep the soil very moist and thus provide optimum conditions for infection. No infection occurred within 45 days after inoculation, and the experiment was discontinued.

DISCUSSION

Infection of pink calla plants by *Phytophthora erythroseptica*[1] is chiefly characterized by wilting and subsequent death of the foliage. Apparently the fungus is unable to parasitize the roots and corms. Further, the pink calla isolate also failed to infect white calla plants and rhizomes. In comparison, the white calla isolate was pathogenic to mature rhizomes of white calla, but failure attended efforts to infect the foliage, roots, and rhizomes of actively growing white calla potted plants which had been artificially inoculated in the greenhouse. The white calla isolate also failed to infect pink calla potted plants and corms. Apparently there are distinct strains of the fungus.

Search has been made since 1946 in the white calla areas, during the growing season, for a trace of this disease, but without success. Apparently, it is a minor disease and very restricted in its geographical distribution. Its potentialities as a serious disease should not be overlooked.

Apparently, this is the first record of *Phytophthora erythroseptica* as a pathogen on white calla.

SUMMARY

A rhizome rot of white calla is prevalent in the San Francisco Bay region of California.

External symptoms of the disease consist of small to large, irregular-shaped, dark brown, water-soaked lesions which later become dry and slightly sunken. Internally, infected tissues are firm but rubbery in texture, light gray, and odorless.

The causal organism has been identified as *Phytophthora erythroseptica* Pethyb.

An isolate of the fungus proved pathogenic to healthy white calla rhizomes in greenhouse tests. The incubation period was about 10 days. In pot experiments, the fungus failed to infect the foliage, roots, and rhizomes of actively growing, healthy plants.

DIVISION OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA, AND
DEPARTMENT OF BOTANY, UNIVERSITY OF MISSOURI
COLUMBIA, MISSOURI

THE EFFECT OF FUNGISTATIC AGENTS ON THE BACTERIAL FLORA OF THE RHIZOSPHERE

FRANK E. HALLECK[1] AND VINCENT W. COCHRANE

(Accepted for publication January 30, 1950)

Evidence has been presented in recent years (4, 8, 12) that the number and types of bacteria present in the rhizosphere of crop plants may be causally associated with root rot. Experimental manipulation of the rhizosphere has heretofore been accomplished by adding to the soil certain organic substances which serve as sources of energy or nitrogen or both. This procedure has the disadvantage of affecting all soil microorganisms, whether in the soil of the rhizosphere or in soil more distant from the roots. It has the added disadvantage that the organic supplements may affect root rot directly (3).

The present work describes preliminary experiments designed to test the possibility of influencing the rhizosphere by applying toxicants to the plants. It was expected that some of these, particularly the water-soluble compounds, would be translocated through the plant, as shown for copper by Mader et al. (7), and be lost by the root cells to the soil in some degree. Losses of soluble compounds from roots have been reported occasionally; e.g., nitrogen compounds by Virtanen (11), and *trans*-cinnamic acid and other compounds by Bonner and Galston (2). Substances specifically affecting the rhizosphere microflora have also been reported (6, 9) to be excreted from roots.

If toxic compounds can be so translocated and lost to the soil, there are three possible implications. First, the method might serve for experimental control of the rhizosphere population. Second, fungicides applied routinely for leaf diseases might be studied for their effect on the rhizosphere and, through it, on root rot. Third, the method might be applicable to the study of the process of root excretion.

METHODS

Bean plants 3 weeks old, grown singly in 6-in. pots in a sand-compost mixture in the greenhouse, were dipped in solutions or suspensions of fungicides and allowed to dry in an inverted position; no fungicide reached the soil by drip, runoff, or washing.

After 96 hr. the plants were removed from the pots and all loose soil was shaken from the roots. The roots and closely adherent soil particles were shaken in sterile water, and dilutions were made directly from the resulting suspension of soil particles. Appropriate aliquots were plated in soil extract-mannitol agar (10) at pH 7.8–8.0, and bacterial colonies were counted after 7 days at room temperature. All counts were converted to

[1] Present address: Food Technology Department, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

numbers per gram of rhizosphere soil, the latter obtained by drying and weighing the soil in the initial suspension.

Control soil was obtained from the same pot by sampling areas of soil to which the bean roots had not penetrated.

Untreated plants were handled in the same way; i.e., a rhizosphere and a control sample were taken as described for the treated plants.

No attempt was made to count fungi, since there were in any case not many present, and since in addition sporulation of soil fungi is common enough to make colony counts almost meaningless.

Soil moisture was only approximately controlled, and some error is thereby introduced, since the moisture level is a factor in determining the amount of soil retained by shaken roots.

TABLE 1.—*The effect of fungistatic agents on the number of bacteria in the rhizosphere of bean plants*[a]

Fungistatic agent	Concentration	Ratio: Bacteria in rhizosphere / Bacteria in control soil		Percentage change in ratio caused by toxicant
	gm. per L.	*Treated*	*Not treated*	
Bordeaux	0.2[b]	9.7	20.3	−52.2
Tetramethylammonium bromide	0.5	8.0	3.6	+122.2
Cetyltrimethylammonium bromide	0.1	8.8	5.0	+76.0
Spergon	1.31	12.0	3.6	+233.3
Phygon-XL	1.77	14.9	3.6	+313.9
Dithane Z-78	1.88	6.9	10.6	−34.9
Proflavine	0.02	11.2	4.4	+154.5
Malachite green	5.0	29.8	46.5	−35.9

[a] Counted in dilution plates of rhizosphere and control soil collected 96 hr. after application of toxicant.
[b] As metallic copper.

Of the materials used, the quinones Spergon (wettable) and Phygon-XL, and the carbamate Dithane Z-78 are standard commercial fungicides. Standard Laboratory Bordeaux (1) was prepared with a copper content of 0.02 per cent. Tetramethylammonium bromide and cetyltrimethylammonium bromide belong to the group of cationic detergents known to be both bacteriostatic and fungistatic (5). Malachite green is a fungistatic dye (5), and proflavine, also a dye, has been shown (unpublished) to be inhibitory to mycelial growth and spore germination of *Sclerotinia fructicola*. All materials were used at concentrations known from the literature to be nonphytotoxic, or so determined by preliminary experiments in which visible leaf lesions were the criterion of injury.

RESULTS AND DISCUSSION

The data from a series of tests are in table 1. Each figure represents the mean of three replicates. Absolute numbers of bacteria and, to a lesser

extent, the ratio of rhizosphere to control soil bacteria fluctuated widely, the primary influence apparently being seasonal. For this reason, the ratio of rhizosphere to control counts is the only figure used. Calculation of the percentage change attributable to the fungicide is included, a positive number meaning that the treatment increased the relative number of rhizosphere bacteria.

Bordeaux mixture, as expected, caused a sharp reduction in the ratio, i.e., decreased the number of bacteria in the rhizosphere relative to the control soil. That this was the result of copper reaching the soil from the roots can be inferred also from chemical analyses, which indicated an increase in the copper content of the rhizosphere soil of approximately 60 per cent.

Malachite green and Dithane Z-78 exerted an effect comparable to that of Bordeaux in direction but of smaller magnitude, each causing approximately a 35 per cent change in the ratio of rhizosphere to control soil bacteria.

These three compounds thus yielded data in accordance with the original hypothesis; all reduced the rhizosphere population. Both copper and malachite green may have passed through the plant unchanged and been excreted by the roots in toxic amounts. In view of the limited solubility of Dithane Z-78 as a compound, some fragment, possibly zinc or a soluble breakdown product of the organic moiety, must be postulated as the active material.

All of the remaining materials had the opposite effect: more bacteria appeared in the rhizosphere of treated plants (relative always to the number in control soil from the same pot) than in untreated plants. The disparity of ratios is particularly large in the cases of Spergon and Phygon-XL, smaller in the cases of proflavine and the cationic detergents.

Two possible explanations may be offered for the unexpected effect of this last group of materials. One, probably the less likely, is that very small amounts of a toxic compound reached the rhizosphere zone but the concentration was in a range actually stimulatory to at least some microorganisms. This explanation cannot be ruled out, but it seems unlikely that such diverse materials should have the same effect, an effect not certainly known for any compound under more fully controlled *in vitro* conditions, so far as growth is concerned.

The more likely explanation, and the one provisionally suggested, is that these materials cause injury to the plant in some way, without visible symptoms, and the result of this injury is an increased loss of soluble carbon compounds, amino acids, or growth factors from the root cells to the adjacent soil and soil solution. Increased growth of soil bacteria results, on this hypothesis, from the increased concentration of nutrients made available.

SUMMARY

Preliminary studies of the effect of fungistatic materials applied to the leaves of bean plants demonstrate that Bordeaux mixture, malachite green, and Dithane Z-78 reduce the relative numbers of bacteria in the rhizosphere.

It is suggested that the toxicants or fragments or breakdown products of them are translocated to the roots and lost to the soil solution in amounts inhibitory to some bacteria.

Spergon, Phygon-XL, cetyltrimethylammonium bromide, tetramethylammonium bromide, and proflavine have the opposite effect, in varying degrees, increasing the relative number of rhizosphere bacteria. A provisional explanation of these findings is that these agents cause some injury to the plant, and the injury results in increased root excretion of compounds which serve as nutrients for rhizosphere bacteria.

WESLEYAN UNIVERSITY

MIDDLETOWN, CONNECTICUT

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ORGANIC VS. COPPER FUNGICIDES FOR CONTROL OF MELANOSE

JAMES F. L. CHILDS[1]

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In 1896 the name melanose was used by Swingle and Webber to describe the disfiguring lesions of *Phomopsis citri* on citrus fruit and foliage (7). They showed that melanose could be controlled by two applications of 6–3½–80 Bordeaux mixture. However, spraying for control was seldom practiced, because the population of scale insects increased after applications of Bordeaux mixture. Later it was demonstrated that 1 percent of oil with 6–6–100 Bordeaux would prevent the increase of scale insects and give good commercial control of melanose and white fly (9). Growers were quick to use the new formulation, but it was soon discovered that there were certain disadvantages in the Bordeaux-oil combination. Fruits and even leaves were occasionally scarred (copper-oil injury and star-melanose) through the use of the combination (4, p. 177) and, furthermore, a second and occasionally even a third application of oil were required for control of scale insects. Since that time it has been learned that almost any material, even road dust, that deposits a particulate residue on the foliage contributes to increasing the scale population (2, 3, 6).

The inconspicuous residue reported for some of the organic fungicides (1) prompted a series of experiments to compare the melanose-controlling ability of a number of organic and quasi-organic fungicides with that of several copper compounds. When compared on the basis of equal metallic copper content, the copper fungicides Yellow Cuprocide, copper-oxychloride sulfate, Copofilm, and Bordeaux mixture are reported (8) to provide equally good control of melanose.

MATERIALS AND METHODS

The following 28 materials were used in one or more tests:

Dithane D-14 and HE-175, disodium ethylene bisdithiocarbamate
Phygon, 2,3 dichloro-1,4-naphthoquinone
Fermate, ferric dimethyl dithiocarbamate
Compound 341, mixture of glyoxalidines, containing 2-heptadecyl, 2-heptadecenyl, and pentadecyl glyoxalidines
Compound 337, 1-hydroxyethyl-2-heptadecylglyoxalidine
8-Hydroxyquinoline sulfate
Good-rite P.E.P.S., polyethylene polysulfide, alone, and as a sticker for other fungicides
Compound 169, copper zinc chromate complex
Bional A, 25 per cent solution of high molecular alkyl-dimethyl benzylammonium chlorides
Bional, ST, ethyl ammonium bromide
MB-584, zinc mercaptobenzothiazole
MB-459, zinc ethylene bis(N-2 cyanoethyl)dithiocarbamate
MB-583, dicyclopentamethylene thiuramdisulphide
MB-358, copper mercaptobenzothiazole

[1] Pathologist, Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

Preventol GD, 2′,2″-dihydroxy-5′,5″-dichloro diphenyl methane
Compound 918, copper salt of phenylmercaptoacetic acid, 29 per cent
Compound 308, copper nitrodithioacetate, 66 per cent active material
Compound 1609B, silver ammonium complex of succinic acid anhydride (0.55 per cent metallic Ag.)
658 (Batch 10), copper-zinc chromate complex
Puratized N5D8, phenylmercurietrihydroxyethylammonium lactate
Cu(93–22)36, $CuNH_3$ salt of succinic acid anhydride (9.3 per cent Cu)
Copper 8-Quinolinolate
Copper A, tetracopper calcium oxychloride
Yellow Cuprocide, yellow cuprous oxide
Micronized copper oxide (cuprous), average particle size, 0.8 μ
Microatomized copper oxide (cuprous), average particle size, 3.5–5.0 μ
Tribasic Copper Sulfate, 52 per cent Cu
Bordeaux mixture

In general the concentrations of fungicide were those recommended by the manufacturer. Sprays were applied late in April or early in May, depending on the season and time of blooming. March and April are generally dry months in Florida, with little melanose development. Favorable conditions for melanose (rain and high humidity on three successive days) usually commence in May but may not appear until as late as June.

Grapefruit trees were selected for the experiments because they are more susceptible to *Phomopsis citri* than are the sweet oranges and tangerines, and because the melanose spots are more easily distinguished on the yellow grapefruit rind. Blocks of mature trees 15 or more years of age were selected because trees of that age are virtually certain to have a sufficient number of dead twigs to provide for the production of abundant spores of *P. citri* and so constitute a good test. In all cases the spray blocks were surrounded on at least three sides by other citrus trees of comparable age.

In the first experiments each treatment was applied to eight trees in a row, but in later experiments the number of trees was reduced to six when analysis of the results indicated that the latter number was adequate.

In October, sometimes later, samples of 50 fruit were picked at eye level from the perimeter of each tree in a treatment. The fruit from each tree was handled as a separate unit. After washing, and gassing with ethylene to destroy the remaining chlorophyll and to intensify the yellow color of the rind, the fruits were graded into four classes or levels of melanose infection. Grading was accomplished by counting the number of spots in one square inch on the cheek of each fruit at a site of average density of melanose spots on that fruit. Ruehle's scale (5, pp. 17, 18) of melanose infection levels, giving the number of spots per sq. inch, was adopted and for statistical analysis an arbitrary factor was selected for each level: Very slight to none, 0 to 10 spots (Factor 1); slight, 11 to 25 spots (Factor 12); moderate, 26 to 50 spots (Factor 27); severe, 51 or more spots per sq. inch (Factor 52).

For the purpose of comparing the various experiments, all treatments were reduced to the basis of a 300-fruit sample. Then in order to simplify the comparison of treatments, the numbers of fruit in each infection level

were reduced to a single figure or index for each treatment. That index was calculated in the following manner: For each treatment the number of fruit in each infection level was multiplied by the factor arbitrarily chosen for that level. The sum of the products constitutes the melanose index for that treatment. Example (1948 spray trial on Marsh grapefruit, treatment No. 1 (MB-584)): There were 0 fruits in lowest infection class, 130 fruits in slight melanose class, 88 fruits in moderate melanose class, and 82 fruits in severe class; total, 300 fruits. Then $0 \times 1 = 0$, $12 \times 130 = 1560$, $27 \times 88 = 2376$, and $52 \times 82 = 4264$; total, 8200 (see Table 4).

RESULTS

1946 Trial: In this trial six organic fungicides were compared with Bordeaux mixture. Sprays were applied May 4 and 5, 1946, to Marsh grapefruit trees that had been in full bloom on March 8. Fruits were picked and graded, and the melanose indices were calculated. The results (Table 1) indicate that there was virtually no significant difference due to treatment between the untreated check and the six organic fungicides: Compound 341, Compound 337, 8-Hydroxyquinoline sulfate, Dithane, Phygon, and Fermate. Bordeaux mixture gave excellent commercial control in that practically the entire sample of fruit graded into the two lowest classes of melanose infection, with a melanose index of 3,326, as compared with the unsprayed check with a melanose index of 14,365. If the total fruit sample should fall into one of the four levels of infection the melanose index for the respective levels would be (a) 0–10 spots per sq. inch, index 300; (b) 11–25, 3,600; (c) 26–50, 8,100; and (d) 51+, 15,600.

TABLE 1.—*Results of 1946 spray applications to Marsh grapefruit*

Spray material	Conc. per 100 gal.	Melanose index[a]
Compound 341 + wettable sulfur	1 lb. + 5 lb.	14,168
Do 337 + do	1 lb. + 5 lb.	13,908
8-Hydroxyquinoline sulfate	13 lb.	13,925
Bordeaux mixture	6 lb. : 4 lb.	3,326
HE-175 + $ZnSO_4$ + Ca(OH)	1 lb. + 1 lb. + ½ lb.	13,657
Phygon	1 lb.	14,865
Fermate	1 lb.	13,800
Check	.....	14,365

[a] The melanose index figure was arrived at by segregating the 300-fruit sample into four levels of infection: fruit with (a) 0–10 spots per sq. in., (b) 11–25 spots, (c) 26–50 spots, and (d) 51 + spots. The number of fruits in the (a) group was multiplied by 1, in the (b) group by 12, in the (c) group by 27, and in the (d) group by 52. The sum of the products of the four groups is the melanose index figure. If the total fruit sample should fall into one of the four levels, the melanose population figure for the respective levels would be (a) 300, (b) 3,600, (c) 8,100, and (d) 15,600.

L.S.D. at 5 per cent level, 493; at 1 per cent level, 649.

1947 Trial: The fungicidal properties of eight compounds were compared in two experiments on Marsh grapefruit trees. In the first experiment the trees were in full bloom on April 9 and were sprayed April 28. Of the five

treatments applied, Bordeaux mixture was the only one in which the melanose index was less than 3,600 (Table 2). The melanose index in the unsprayed check plot was 7,524. The second best material in the test was copper 8-hydroxyquinoline (index 5,824). The Phygon and the Dithane (D-14) treatments, with and without polyethylene polysulfide, were poorer

TABLE 2.—*Results of 1947 spray applications to Marsh grapefruit, first experiment*

Spray material	Conc. per 100 gal.	Melanose index[a]
Good-rite P.E.P.S.	2½ lb.	14,245
Do + Phygon	2½ lb. + 1½ lb.	8,820
Do + Dithane (D-14)	2½ lb. + 1½ lb.	10,221
Do + Cop. 8-Quinol.	2½ lb. + 1½ lb.	5,824
Phygon + Vatsol O. T.	1½ lb. + 100 gm.	10,940
Dithane (D-14) + Vatsol O. T.	1½ lb. + 100 gm.	9,364
Bordeaux + Vatsol O. T.	6 lb. : 4 lb. + 50 gm.	3,173
Check		7,524

[a] See footnote to table 1.
L.S.D. at 5 per cent level, 695; at 1 per cent level, 914.

than the untreated check lot, though not significantly so. Probably the polyethylene polysulfide spreader did not significantly affect the efficacy of the Dithane or Phygon treatments, but it is interesting to note the high level of the melanose population obtained with this material alone (index 14,245).

In the second experiment Bordeaux mixture was again outstanding, with an index of 811 (Table 3), as compared with the two unsprayed checks

TABLE 3.—*Results of 1947 spray applications to Marsh grapefruit, second experiment*

Spray material	Conc. per 100 gal.	Melanose index[a]
Dithane (D-14) + $ZnSO_4$ + $Ca(OH)_2$	1½ lb. + 1 lb. + ½ lb.	10,620
Phygon + do + do	1½ lb. + 1 lb. + ½ lb.	10,715
Preventol GD + do + do	1½ lb. + 1 lb. + ½ lb.	10,830
Copper 8-Quinol. + do + do	1½ lb. + 1 lb. + ½ lb.	5,863
Phygon + B-1956	1½ lb. + 2 oz.	6,589
Bordeaux + Vatsol O. T.	6 lb. : 4 lb. + 0.4 per cent	811
Check		5,956
Phygon + Vatsol O. T.	1½ lb. + 0.5 per cent	6,277
Copper A compound + DuPont sticker	1½ lb. + 4 oz.	2,093
Compound 341 + $Ca(OH)_2$ + Vatsol O. T.	2 qt. + 3 oz. + 0.25 per cent	6,178
Check		7,113
Phygon + Vatsol O. T.	½ lb. + 0.5 per cent	6,902
Do + do	1 lb. + 0.5 per cent	5,266
Do + do	2 lb. + 0.5 per cent	7,345

[a] See footnote, table 1.
L.S.D. at 5 per cent level, 281; at 1 per cent level, 370.

(indices 5,956 and 7,113). The second best material was Copper A compound, with an index of 2,093. In a previous test Phygon had shown some promise of melanose control, hence it was applied here at several concentrations and also in one treatment with zinc sulfate-hydrated lime sticker. However, none of the various formulations of Phygon gave a

satisfactory degree of control. The same was true of the organic materials, Preventol GD, Compound 341, and Dithane D-14.

1948 Trial: Two experiments were set up, the first being applied to Marsh grapefruit trees, and the second to the Duncan variety of grapefruit. Both blocks of trees were in full bloom on March 15 and both were sprayed during the period April 19–23. Fruit samples were picked October 25 to November 6, washed, and graded.

In the Marsh grapefruit block the melanose indices of the two checks were high—8,145 and 7,540 (Table 4), indicating a good test of fungicidal

TABLE 4.—*Results of 1948 spray applications to Marsh grapefruit*

Spray material	Conc. per 100 gal.	Melanose index[a]
MB-584	3 lb.	8,200
MB-459	1 lb.	8,041
MB-583	1 lb.	7,206
Micronized copper oxide + Vatsol O. T.	2 lb. + 100 gm.	2,146
Bordeaux mixture + Vatsol O. T.	6 lb. : 4 lb. + 100 gm.	5,780
Compound 169	2½ lb.	5,677
Check		8,145
Bional A	¼ gal.	8,435
Bional ST	1 lb.	8,009
Yellow cuprocide	2 lb.	5,336
Cu(93-22)36 + Filmfast	2½ lb. + ½ lb.	9,288
Check		7,540
Compound 918 + Filmfast + Vatsol O. T.	2 lb. + ½ lb. + 70 gm.	8,830
Compound 308 + do + do	2¼ lb. + ½ lb. + 70 gm.	8,770
Compound 1609B + do + do	1 pt. + ½ lb. + 80 gm.	9,010
Puratized N5DS + Vatsol O. T.	½ gal. + 80 gm.	5,179
Tribasic copper sulfate (52 per cent Cu) + Vatsol O. T.	3 lb. + 80 gm.	5,914

[a] See footnote, table 1.
L.S.D. at 5 per cent level, 714; at 1 per cent level, 939.

effectiveness. Micronized cuprous oxide was outstandingly the most satisfactory (index 2,146). Puratized N5DS (index 5,179) and Yellow Cuprocide (index 5,336) tied for second place, and Compound 169 (index 5,677) and Bordeaux (index 5,780) tied for third place. The melanose index for Bordeaux and that for tribasic copper sulfate (index 5,914) were similar, which tends to bear out the previously mentioned observation that those two materials give equally good control when compared on the basis of their metallic copper content. Three fungicides, Micronized cuprous oxide at 2 lb., tribasic copper sulfate at 3 lb., and Bordeaux mixture at 6–4–100, had a metallic content of 1.58 lb., 1.56 lb., and 1.53 lb. per 100 gal., respectively.

In the Duncan grapefruit block, a low level of melanose infection was apparent from the melanose index figures of the two checks—3,629 and 3,315 (Table 5). Such a low level leaves very little room for improvement or for comparison of treatments and indicates that escape due to unfavorable weather (pathologically speaking) was probably a factor. As a result, the differences between treatments were less distinct, but probably not with-

out value. It will be sufficient to point out that among the materials giving the lowest melanose index figures were (a) tribasic copper sulfate, (b) Yellow Cuprocide, (c) Compound 658 (copper zinc chromate complex), (d) Compound 308 (copper nitrodithioacetate), (e) Phygon, (f) Compound 918 (copper salt of phenylmercaptoacetic acid), (g) Bordeaux mixture, and (h) Microatomized cuprous oxide.

DISCUSSION AND CONCLUSIONS

Bordeaux mixture has been the yardstick by which the materials in these tests were judged. Any compound that gave control as good as that rendered by Bordeaux mixture (6–4–100) was judged to be a satisfactory fungicide unless found to be deficient in some other regard, such as exces-

TABLE 5.—*Results of 1948 spray applications to Duncan grapefruit*

Spray material	Conc. per 100 gal.	Melanose index[a]
Phygon XL + Vatsol O. T.	1 lb. + 40 gm.	1,837
MB-459 + do	2 lb. + 60 gm.	2,859
MB-583 + do	2 lb. + 60 gm.	2,574
Microatomized copper oxide + Vat. O. T.	2 lb. + 100 gm.	2,203
Bordeaux mixture + Vatsol O. T.	6 lb. : 4 lb. + 100 gm.	2,040
Check		3,629
Compound 169 + Vatsol O. T.	2½ lb. + 100 gm.	4,141
MB-358 + Vatsol O. T.	2 lb. + 100 gm.	3,590
Bional ST	1 lb.	3,641
Copper 8-Quinol. + Vatsol O. T.	1¾ lb. + 100 gm.	3,185
Cu(93-22)36 + Filmfast + Vatsol O. T.	1050 cc. + ½ lb. + 100 gm.	2,401
Check		3,315
Compound 918 + Filmfast	11 oz. + ½ lb.	2,025
Compound 308 + Vatsol O. T.	3 lb. + 80 gm.	1,482
Yellow cuprocide + Vatsol O. T.	1 lb. + 100 gm.	1,497
Bional A	¼ gal.	2,508
Compound 658 + Batch 10	2½ lb. + 100 gm.	1,202
Tribasic copper sulfate (52 per cent Cu) + Vatsol O. T.	1½ lb. + 100 gm.	1,241

[a] See footnote, table 1.
L.S.D. at 5 per cent level, 375; at 1 per cent level, 493.

sive deposit of residue or poor resistance to weathering. As a class, the organic materials were found to be inconsistent as fungicides. For example, MB-583 (dicyclopentamethylene thiuramdisulphide) rated rather close to Bordeaux mixture as regards melanose control in table 5, but in table 4 nearly all the fruit treated with MB-583 were rated third class. The same is true of Compound 918 (copper salt of phenylmercaptoacetic acid); i.e., its melanose control was rated as good as Bordeaux mixture or Microatomized cuprous oxide (Table 5), but fruit sprayed with Compound 918 were rated third and fourth class (Table 4). The same situation is also true of Compound 308. Puratized N5DS gave satisfactory control in the 1948 Marsh grapefruit experiment, but in an earlier test (1945) on single grapefruit trees Puratized N5E at the rate of 1 gal. per 100 gave results only slightly better than the unsprayed control; considerable defoliation, as expected, was noted at that concentration. In that same test 8-hydroxyquino-

line sulfate at the rate of 32 lb. per 100 gal. was slightly better than Bordeaux mixture at 6–4–100 concentration.

As a class, the inorganic fungicides were superior to the organic compounds, and of these the relatively simple copper compounds seemed more consistent than the more complex compounds. For example, Bordeaux mixture, tribasic copper sulfate, and the cuprous oxides have consistently given good to excellent control. The copper zinc chromate complex (Compound 169) gave satisfactory control in the Marsh block in 1948 (Table 4), but in the Duncan block of the same year (Table 5) it had the highest melanose population figure of any material in the experiment. At the same time, Compound 658, another copper zinc chromate complex, gave good control.

As mentioned in the introduction, the purpose of this work was to search for a fungicide with a low level of residue comparable with the best organic materials and equal to Bordeaux mixture as regards control of melanose. The goal was not achieved, in the author's opinion, by any of the organic compounds in the test. On the other hand, the cuprous oxides gave consistently good control of melanose, often exceeding Bordeaux mixture in that regard. In the past, growers and grove caretakers have criticized the cuprous oxides because the residue deposited is so inconspicuous as to make it difficult to determine whether the foliage has been properly covered with spray. As a matter of fact, with cuprous oxide at a concentration of 2 lb. per 100 gal. it would be impossible to determine by inspection where the spray has been applied except for the residue deposited from droplets at the margins and tips of leaves. This apparent disadvantage is really an advantage from the standpoint of lessening scale build-up, and suggests that more attention should be paid to the cuprous oxides by the citrus grower. Of the organic materials used in these experiments, N5DS appears to be the only one worthy of further trial against melanose.

U. S. SUBTROPICAL FRUIT FIELD STATION
ORLANDO, FLORIDA

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POSSIBLE MECHANISM OF VARIATION IN THE IMPERFECT STAGE OF SCIRRHIA ACICOLA

PAUL V. SIGGERS

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During a study of the effect of environmental factors on germination of conidia of the fungus causing the brown-spot needle blight of pines, *Scirrhia acicola* (Dearn.) Siggers,[1,2] germ-tube fusions between different conidial cells, hyphal fusions, and a breaking up of the multicellular conidia along the septa were observed. Details of the observations are reported herein, as of interest to those involved in studies of variation in fungi. In this connection, Hansen and Smith demonstrated hyphal fusions and nuclear migrations through the fusion hyphae of *Botrytis cinerea* Pers. (*B. vulgaris* Fr.) and gave an explanation for the variation in monosporous cultures of this fungus.[3]

Monosporous conidial cultures on agar drops in van Tieghem cells were used in this study. Culture media used consisted of 15 gm. agar containing either malt, potato-dextrose, prune extract, or a decoction of pine needles and malt, with sufficient distilled water to make up 1000 cc. An electrically controlled refrigerator was used for storing the cultures at approximately 41° F.

Germ-tube fusions between different conidial cells. A fruiting body from a pine needle from Louisiana was the source of a monosporous conidial culture that was started September 19, 1937, and placed in a refrigerator 3 days later. Conidia from this culture were transferred in mass to a glass slide and germinated in pine-needle decoction water at 68°–78° F. in November. Figure 1, B shows germ-tube fusion that took place in 40 hr. between two cells of the same spore and apparently between two cells of different spores.

Hyphal fusions. A monosporous conidial culture was started in July 1937 from a stock culture of a strain of the brown-spot fungus from Florida. In 12 days hyphae reached the margin of the agar and started over the lower surface of the glass cover slip (Fig. 1, E). The same hyphal field, photographed again 5 days later, shows that many hyphal fusions had occurred in the marginal zone of growth (Fig. 1, F). In a second single-spore culture, anastomoses apparently took place on the agar surface between hyphae from different cells of the same spore. A photomicrograph of the hyphae in the mesophyll of a pine needle indicates that anastomosis occurs also in nature.

Break-up of multicellular conidia at the septa. Conidia of *Scirrhia acicola* are multicellular and normally 3-septate (Fig. 1, A). At the time

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[2] Siggers, Paul V. The brown spot needle blight of pine seedlings. U. S. Dept. Agr., Tech. Bul. 870. 1944.

[3] Hansen, H. N., and Ralph E. Smith. The mechanism of variation in imperfect fungi: *Botrytis cinerea*. Phytopath. **22**: 953–964. 1932.

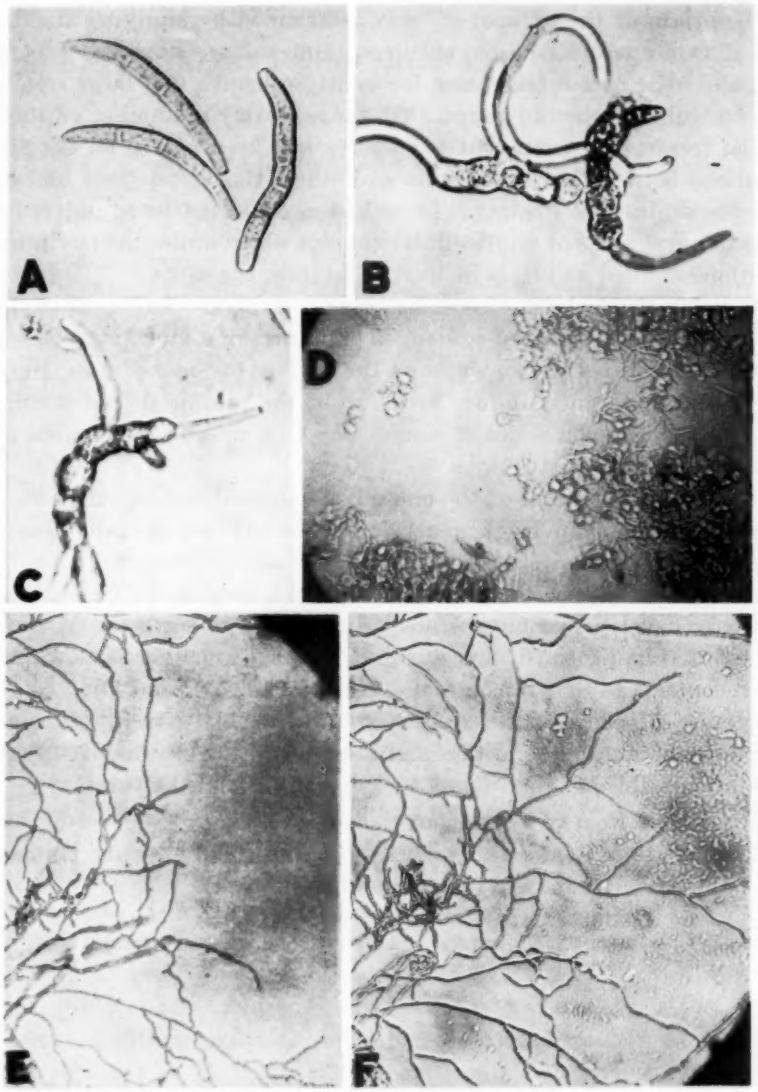

FIG. 1. A. Nongerminated conidia of *Scirrhia acicola* from a monosporous culture. B. Germinating conidia; note the inactive cell of the horizontal spore, and germ-tube fusion between the swollen second and fourth cells. Apparently fusion between two cells of different spores has occurred also. C. Conidium germinating on potato-dextrose agar after 48 hr. at 30° C. D. Small spheroid cells apparently formed by the rounding up and separation of individual conidial cells at the septa. Germ tubes developed in a month-old stock culture at cold storage. E. Hyphae on the lower surface of a glass cover slip, August 12, 1937. F. The same microscope field, photographed later, shows that numerous hyphal fusions have occurred in the marginal zone of growth.

of germination the spores enlarge and germ tubes develop from individual cells of a spore—generally one germ tube to a cell (Fig. 1, C). In May 1937 a transfer from a month-old test-tube stock culture in cold storage showed masses of what appeared to be a new kind of spore (Fig. 1, D), possibly a contamination.

The origin of these "spores" was determined by studying the development of monosporous conidial cultures. One culture started September 19, 1937, and kept in a refrigerator for nearly a month had large numbers of these one-celled, spheroid bodies. On the same day in another culture, with parallel treatment except that two spores had been placed on the agar, all gradations between normal conidia and others that apparently had divided along the septa were present. These bodies could not be considered spores but as integral parts of multicellular conidia which under the environmental conditions divided at places of least resistance, the septa.

Probably the division of conidia into several cells can be explained on a physical basis as a result of prolonged exposure, in a vibrating refrigerator, to low temperatures and to water on the surface of the medium. Since conditions that apparently induce break-up of the conidia do not occur in nature, the phenomenon is not of significance as a possible mechanism of variation in the brown-spot fungus.

In June 1937, several of the one-celled spheroid bodies were transferred as single spores to van Tieghem cells and then to tubes of malt-extract agar. Some of the cultures were examined after 6 months' storage in a refrigerator. No spores or sporelike bodies were found, despite the fact that conidia commonly develop in monosporous cultures in 3 to 4 weeks. An upraised mycelial mat had formed in each tube. Hyphal growth was slow and toruloid in contrast to that in conidial cultures (Fig. 1, E and F).

Cytological and cultural studies would probably give interesting information on the nuclear condition and behavior of the brown-spot fungus and explain the failure of the fungus to sporulate in some cultures.

DIVISION OF FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING
U. S. DEPARTMENT OF AGRICULTURE
SAUCIER, MISSISSIPPI

STUDIES ON STRAINS OF CUCUMBER VIRUS 1 FROM SPINACH[1]

JOSEPH P. FULTON

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INTRODUCTION

Eight virus diseases have been reported from field-grown spinach (*Spinacia oleracea* L.) in the United States. These eight are cucumber mosaic (cucumber virus 1), aster yellows, sugar-beet curly top, beet mosaic, spotted wilt, spinach yellow dwarf, western cucumber mosaic, and celery calico (5, 6). Cucumber virus 1 (1), originally called spinach blight on this host (3), has been considered the predominant virus disease of spinach in Arkansas (2). Since diseased plants, observed in fields in Arkansas, vary considerably in symptom expression, the question arises as to whether one or more viruses may be responsible for the damage to spinach in this State. The present work is a study of six different virus isolates obtained from field-grown spinach. The identification of these samples as strains of cucumber virus 1 and the reactions of varieties of spinach are described.

MATERIALS AND METHODS

Spinach plants with viruslike symptoms were collected from various fields near Van Buren, Arkansas. Greenhouse plants of tobacco (Havana Seed 38), cucumber (Straight 8), cowpea (Blackeye), and spinach (Giant Noble) were inoculated with material from each specimen. The inoculum was prepared by grinding some of the specimen in 5 ml. of 0.1M Na_2HPO_4. A gauze pad was saturated with this inoculum and wiped on leaves of plants which had been previously dusted with carborundum. Plants were rinsed with water following inoculation. Greenhouse temperatures fluctuated between 72° and 78° F.

Twenty-six virus isolates were obtained in this manner, all of which were presumed to be strains of cucumber virus 1. From this group, six isolates, designated A, B, C, D, E, and F, were selected for more detailed study. Preliminary observations indicated that the six would show a variation in symptom expression. These isolates were maintained on young tobacco plants (var. Havana Seed 38 and the Hybrid, *Nicotiana tabacum* × *N. glutinosa*). Inoculum for additional studies was prepared from these young infected tobacco plants in the manner outlined above.

These six virus isolates were used in the condition in which they were obtained from field-grown spinach, no special methods of purification being undertaken. In the course of the study no evidence was obtained to indicate that any isolate was a mixture of two or more strains, although mixtures

[1] Research paper No. 927, Journal Series, University of Arkansas. Published with the permission of the Director of the Arkansas Agricultural Experiment Station.

have been obtained from field-grown spinach in the course of the work. Reinoculations of these isolates from selected host plants always produced symptoms characteristic for the original isolate. Two variations, which were not interpreted as indicating a mixture, were noted. One was the development of an occasional yellow spot on tobacco leaves infected by isolates A and D, from which yellow variants could be obtained. One reinoculation of strain C from an infected spinach plant produced symptoms typical for the original isolate on most hosts but much milder than the original isolate on spinach. In the remainder of this paper the six original isolates will be referred to as strains.

REACTIONS OF VIRUS STRAINS ON SELECTED HOST PLANTS

Symptoms produced by each of the strains on selected hosts are in table 1. Five or more plants of each species were inoculated in each trial, and each trial was repeated two or more times. In addition to the symptoms in table 1, the following were observed on these additional hosts:

Amaranthus caudatus L. (*love-lies-bleeding*): All strains produced necrotic local lesions and necrosis along the veins of inoculated leaves. No systemic symptoms developed except in the case of strain B, where an occasional plant showed twisting and distortion of new leaves.

Beta vulgaris L. (garden beet var. Early Wonder): Diffuse chlorotic-to-yellow spotting developed on the inoculated leaves with all strains. Spots were most prominent with strain C. With strain E the yellowing appeared as streaks along the veins. No systemic symptoms appeared.

Chenopodium album L. (lambsquarters): All strains produced small, chlorotic spots on the inoculated leaves. No systemic symptoms appeared.

Zea mays var. *rugosa* Bonsf. (sweet corn var. Golden Cross Bantam): A systemic, chlorotic-to-yellow streaking developed with all strains.

Zinnia elegans Jacq. (zinnia var. Lilliput): A systemic, green mottle was produced by all strains except strain C which produced prominent, necrotic, local lesions and no systemic symptoms.

On the basis of symptoms produced, strains C and E could be readily distinguished from each other and from the remainder of the strains. Strain C was characterized by its lethal effect on cowpeas and the production of local lesions on zinnia. Strain E was characterized by the production of local lesions on cowpeas and on garden pea and its inability to infect garden bean. Strains A, B, D, and F differed from each other primarily in the severity of symptoms produced. The reaction of strain F on garden pea was consistently more severe than that of the other strains on this host. Strain B produced milder symptoms than the other strains on most host plants. No difference between strains A and D was evident in the reactions on these host plants. A difference will be noted in the reactions of these two strains on spinach.

TABLE 1.—*Local and systemic symptoms produced by various strains of cucumber virus 1 on selected hosts in the greenhouse*

Species and variety of plant inoculated	Local (L) and systemic (S) symptoms produced by strains[a]				
	A	B	C	E	F
Beta vulgaris var. *cicla* L. (Swiss chard var. Lucullus)	L: fine, diffuse chlorotic spotting. S[b]: mottle and leaf distortion.	L: similar to A. S[b]: similar to A.	L: yellow spotting and streaking. S: none.	L: similar to A. S[b]: similar to A.	L: similar to A. S[b]: similar to A.
Cucumis sativus L. (cucumber var. Straight 8)	L: chlorotic spotting on cotyledons; later disappears. S: vein clearing and green mottle.	L: similar to A. S: similar to but milder than A.	L: similar to A. S: bright yellow mottle.	L: similar to A. S: similar to A.	L: chlorotic spotting, then yellowing and death of cotyledon. S: similar to A.
Lycopersicon esculentum Mill. (tomato var. Rutgers)	L: none. S: vein clearing, mottle, and "fern leaf."	L: none. S: mild distortion of leaves.	L: none. S: stunting, yellowing and mottling; some necrotic spotting and distortion.	L: none. S: similar to B.	L: none. S: mild mottling and leaf distortion.
Nicotiana tabacum L. (tobacco var. Havana Seed 38)	L: none. S: vein clearing and green mottle.	L: none. S: similar to but milder than A.	L: none. S: bright yellow mottle.	L: none. S: similar to A.	L: none. S: similar to A.
Phaseolus vulgaris L. (garden bean var. Landreth)	L: none. S: vein banding, downward curvature of leaves, slight mottle.	L: none. S: similar to but milder than A.	L: death of inoculated leaf. S: death of growing point, stem necrosis.	L: none. S: none.	L: none. S: similar to but more severe than A.
Pisum sativum L. (garden pea var. Alaska)	L: none. S: wilting of lower leaves, mottle of new leaves.	L: none. S: mild stunting.	L: none. S: yellow vein clearing and mottle, then death of plant.	L: necrotic lesions. S: none.	L: none. S: yellowing, mottling, fine necrotic spotting, then wilting and death of plant.
Vigna sinensis (Torner) Hassk. (cowpea var. Blackeye)	L: indistinct chlorotic spots and necrotic flecks. S: vein clearing, stunting, mottling.	L: similar to A. S: similar to but milder than A.	L: prominent chlorotic spots. S: yellowing of growing tips, stem necrosis, rapid death of plant.	L: necrotic lesions. S: none.	L: similar to A. S: similar to A.

[a] Strain D is omitted from this table; the symptoms produced by it are essentially the same as strain A.

[b] Systemic symptoms appeared on only a few of the inoculated plants.

REACTIONS ON VARIETIES OF SPINACH SUSCEPTIBLE TO CUCUMBER VIRUS 1

Young spinach plants, approximately 1 month old, of seven varieties ordinarily susceptible to cucumber virus 1, were inoculated with each virus strain to determine whether symptoms might vary with the variety of spinach. Varieties inoculated were Giant Noble, King of Denmark, Long Standing Bloomsdale, Dark Green Prickly Seeded, Juliana, Viking, and Viroflay. Each strain produced symptoms which were characteristic on all of these varieties of spinach. Spinach varieties differed only in the amount of stunting which resulted. The difference in symptoms produced by the different strains was much greater than any difference that could be attributed to a difference in the spinach variety. The following is a summary of the symptoms produced on the spinach varieties listed above.

Strain A: Irregular chlorotic areas on inoculated leaves, then vein clearing and mottling of new leaves. The outer leaves yellowed and died and the plants were reduced to groups of distorted, mottled, narrow, "shoestring" leaves.

Strain B: Faint chlorotic areas on inoculated leaves, then vein clearing and mottling of new leaves. The outer leaves yellowed and died and the plants were reduced to rosettes of small, circular, dark-green leaves with little or no distortion.

Strain C: Bright vein clearing of the center of the plants, then yellowing and rapid death of the growing points and yellowing and death of the plants in about 15 days. A few plants survived longer and were reduced to small rosettes of yellow, mottled, "shoestring" leaves. Occasionally yellow spots and streaks appeared on the inoculated leaves.

Strain D: Symptoms were similar to, but a little more severe than, those produced by strain A.

Strain E: Vein clearing of inoculated leaves, then vein clearing and some yellowing and distortion of new leaves. Plants then seemed to recover somewhat but remained stunted; new leaves were small and narrow.

Strain F: Chlorotic spots on inoculated leaves, then yellowing of new leaves and death of outer leaves. The plants later were severely stunted and produced rosettes of small, pointed, curled leaves. Many plants died in 20 to 30 days.

The symptoms on these varieties of spinach, although differing as described, were not sufficiently striking to distinguish the strains except in the case of strain C. The rapid yellowing and death of plants inoculated with strain C were markedly different from the symptom expression for the other virus strains. In all cases 100 per cent infection of these susceptible varieties of spinach was obtained.

REACTIONS OF VIRUS STRAINS ON OLD DOMINION AND VIRGINIA SAVOY SPINACH

The spinach varieties Old Dominion and Virginia Savoy were developed primarily for their resistance to cucumber virus 1 (7). It has been recognized that these varieties are not immune from cucumber virus 1 and that

a certain number of plants will develop symptoms of the disease. This is true in the case of mechanical inoculation in the greenhouse (1) and also of aphid transmission in the field (7). In the present work these two varieties were inoculated with the six strains. In four tests, 103 plants of Old Dominion spinach were inoculated with each of the six strains, and in two tests 36 plants of Virginia Savoy were inoculated with each strain. Strains B and C infected only 8 per cent of the Virginia Savoy plants, but approximately 20 per cent of the Old Dominion. Strains A and E infected about 18 per cent of the Virginia Savoy and 25 per cent of Old Dominion. Strain F, on the other hand, attacked 45 to 50 per cent of both varieties and Strain D was able to infect 100 per cent of the plants of both varieties. It is evident that these strains of cucumber virus 1 varied in their ability to infect the resistant varieties of spinach. The surprising result was that strain D was able to infect 100 per cent of the plants.

Additional inoculations of the varieties Old Dominion and Virginia Savoy with strain D were made to see whether the high percentage of infection could be obtained under varying conditions. Forty-eight Old Dominion plants and 48 Virginia Savoy plants were shaded for 7 days prior to inoculation. An equal number of plants remained on the greenhouse bench. A similar series was run with the plants shaded for 7 days following inoculation. In all cases 100 per cent infection was obtained. A total of 138 Old Dominion plants ranging in age from 20 days to 100 days was inoculated with strain D, and 100 per cent infection was obtained. Symptoms appeared more rapidly on the younger plants.

The symptoms produced by strain D on Old Dominion and Virginia Savoy were more severe than the symptoms produced by this strain on the seven susceptible varieties of spinach. A yellow spotting appeared on the inoculated leaves of resistant varieties in 4 to 6 days; the center of the plant turned yellow, and death of the entire plant usually followed within 15 days. Older plants were often able to withstand the shock of the initial symptoms and the growing points developed into small rosettes of narrow, distorted leaves.

When individual plants of Old Dominion and Virginia Savoy spinach became infected with the strains of the virus other than strain D, the symptoms often were not typical of those previously described on susceptible varieties of spinach, but were similar to those described for strain D in that rapid yellowing and death of the plant usually resulted. A few of the affected individuals developed milder symptoms which included stunting, mottling, and some distortion. With strain E the majority of the affected individuals were of this milder type.

CROSS PROTECTION

The identification of strains of cucumber virus 1 by means of the cross protection test has been demonstrated by Price (4). In the present study all of the six strains were shown to be related to one another and to a known

strain of cucumber virus 1[2] by means of this reaction. Since systemic symptoms as well as necrotic local lesions were produced by different strains of the virus on both cowpea and zinnia, these two hosts were used to study cross protection. The reaction of strain C on zinnia is essentially the same as that reported by Price (4) for his strain 6.

To demonstrate cross protection with the known strain of cucumber virus 1, the primary leaves of young cowpea plants (Blackeye) were inoculated with strains A, B, D, and F. Ten plants were inoculated with each strain. Ten days later when the trifoliate leaves had expanded and showed typical mottling they were inoculated with the known strain of cucumber virus 1. Six leaflets on each plant were inoculated. A group of 10 control plants was similarly inoculated. The control plants developed an average of 191 lesions per leaflet. The plants which had been previously inoculated with the strains listed above developed no lesions. A repetition of this test gave similar results.

Since strain E produced local lesions on cowpea, a similar series of tests was run demonstrating cross protection between it and strains A, B, D, and F. The average number of lesions on the leaflets of the control plants inoculated with this strain was 163. Plants which had been previously inoculated with the four strains listed above developed no lesions with the exception that, in one test, plants previously inoculated with strain B developed 0 to 15 lesions per leaflet when inoculated with strain E.

Strain C developed local lesions on zinnia, and cross protection was demonstrated between this strain and the other strains as well as with the known strain of cucumber virus 1. Six young zinnia plants were inoculated with each of strains A, B, D, E, F, and the known strain. Eighteen days later, when the leaves of these plants were mottled, they, as well as a control of six plants, were inoculated with strain C. Four leaves on each plant were inoculated. On the first trial the control plants averaged 21 lesions per leaf, whereas the plants previously inoculated with the strains listed above developed no lesions. On the second trial the control plants developed an average of four lesions per leaf, whereas all plants which had previously been inoculated with the strains listed above, except strain B, developed no lesions. On the plants previously inoculated with strain B, fourteen leaves developed no lesions and ten developed one to four lesions, or an average of less than one lesion per leaf. It would appear from this as well as the work with cowpea plants that under certain conditions complete protection is not obtained between strain B and other strains of cucumber mosaic.

Cross protection was also demonstrated between strain A and Price's strain number 6 on zinnia. The test was similar to that already described for strain C. Six zinnia control plants and six plants which had been previously inoculated with strain A were inoculated with Price's strain number 6. The control plants developed an average of 17 lesions per leaf, whereas plants which were infected with strain A developed no lesions.

[2] Kindly supplied by Dr. R. W. Fulton.

PROPERTIES

In a series of tests made to determine the thermal inactivation point, greater variation was observed between different tests than between the virus strains. Virus extracts obtained from young infected tobacco plants were placed in 1.3×10-cm. thin-walled test tubes and heated for 10 min. in a water bath controlled at the various temperatures. Cowpea plants were used to test the infectivity of each sample. In a series of tests made during December, January, and February, inactivation of all strains was obtained between 55° C. and 60° C. In one test inactivation was obtained below 55° C. In several tests made during April and May, inactivation was obtained between 65° C. and 70° C. Until a large number of tests can be made considering a variation in source of inoculum and test plants, it must be concluded that the inactivation point for all of these strains is between 65° C. and 70° C. From the tests so far conducted no significant difference between strains was evident from the thermal inactivation data.

The same variation was evident between tests to determine the longevity *in vitro* of the virus strains. The same inoculum used in the thermal inactivation tests was stored at 20° C. Infectivity was lost in 2 to 3 days with samples tested during December, January, and February. Samples obtained in April and May maintained their virulence for 10 days. The greater longevity was correlated with a higher thermal inactivation point. This may have been due to an increased virus content of the extract or to greater susceptibility of the test plants, or to other factors. Here, again, no significant difference was noted between the strains of the virus.

DISCUSSION

This work demonstrates that field-grown spinach in Arkansas is attacked by a variety of strains of cucumber virus 1. Since only six isolates were considered, the range in variability may be greater than that described. No attempt was made to identify these strains with strains described from other host plants. The extreme variability of cucumber virus 1 strains makes such a task beyond the scope of the present work.

The resistance of the spinach varieties Old Dominion and Virginia Savoy was demonstrated in the case of five of the six strains of cucumber virus 1. The work presented indicates a need for additional studies on the nature of the resistance of these spinach varieties. The resistance, apparently, is to the initial infection rather than a type of tolerance or a restricted development due to a local lesion reaction, as is usually the case in resistance to virus diseases demonstrated in other species. In this study the plants which were not infected by the five strains of the virus might be considered immune. The plants which were infected might be considered susceptible and their presence explained on the basis of the heterozygous nature of these varieties of spinach. There are some indications that this may not be the case or at least that these susceptible plants react in a manner which is not typical of the reaction of the susceptible varieties of spinach to cucum-

ber virus 1. Whatever the nature of the resistance of Old Dominion and Virginia Savoy spinach, it has been demonstrated that at least one strain of cucumber virus 1 is capable of breaking down this resistance. This was done, of course, with greenhouse-grown plants using mechanical transmission. The same variation may not exist in the field where the spread of the virus is dependent on aphid transmission. If aphids are related to the susceptibility or resistance of the varieties of spinach, the extent to which they are related and the factors involved should be known, to facilitate additional breeding work with spinach.

SUMMARY

Six isolates of cucumber virus 1 obtained from field-grown spinach were studied in detail. These isolates, designated as strains A, B, C, D, E, and F, produced varying symptoms on selected host plants. Strains C and E were readily distinguished from each other and from the other strains. The remaining four strains differed chiefly in the severity of the symptoms produced.

When the virus strains were inoculated into seven varieties of spinach susceptible to cucumber virus 1, the difference in symptoms produced by the strains was much greater than any variation attributed to a difference in the spinach variety.

The resistance of Old Dominion and Virginia Savoy spinach to five of the six strains of the virus was demonstrated, although a certain percentage of the plants became infected with each strain. These two varieties of spinach were found to be completely susceptible to strain D. The need for additional work on the nature of resistance of these two varieties of spinach is indicated.

The identification of all six isolates as strains of cucumber virus 1 was demonstrated by means of the cross protection test. The thermal inactivation point for all the strains was between 65° C. and 70° C.

AGRICULTURAL EXPERIMENT STATION, UNIVERSITY OF ARKANSAS
FAYETTEVILLE, ARKANSAS

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SERIAL TRANSMISSION OF BEET-MOSAIC VIRUS BY THE GREEN PEACH APHID

EDWARD S. SYLVESTER[1]

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Although beet-mosaic virus, *Marmor betae* Holmes, is a nonpersistent type of virus, its limited retention and serial transmission by infective green aphids, *Myzus persicae* (Sulzer), was demonstrated by Watson.[2] This demonstration is possible if the feeding times involved are kept sufficiently short. The following work is concerned with results of experiments on multiple feeding and serial transmission of the beet-mosaic virus by the green peach aphid.

MATERIALS AND METHODS

The beet-mosaic virus was obtained from infected greenhouse-reared sugar beets. The noninfective aphids were obtained from stock colonies kept for experimentation, and all transfers were made by the camel's-hair brush technique. The test plants were healthy sugar-beet seedlings in the cotyledon stage, and the inoculated plants were fumigated with nicotine before being placed in the greenhouse for incubation. The feeding intervals were timed with a stop-watch.

In studying serial transmission, 30 replications of the experiment were made. Aphids, selected at random from a noninfective population, were placed in a vial for a mean starvation period of 2.2 hr. Following this, one individual for the A series was allowed an infection feeding of 20–30 sec. upon a virus source, then was transferred to a healthy beet seedling for a 10-sec. feeding, then to a second healthy beet for another 10-sec. test feeding. The transfers were repeated until the individual had fed for 10 sec. upon each of ten healthy plants.

The individuals in series B had the same treatment as the aphids in series A, except that the insects, following the initial 20–30 sec. infection feeding, were allowed another separate 10-sec. infection feeding. Following this the insects were given a 10-sec. test feeding on each of nine healthy beet seedlings. In the remaining series, C through J, the infection feedings progressively increased by one 10-sec. period, and the test feedings so decreased by one healthy plant, until the aphids in the last series, J, had an initial 20–30-sec. infection feeding and nine additional 10-sec. infection feedings, and finally were transferred to one healthy beet seedling for a 10-sec. test feeding.

By this method it was hoped to demonstrate that an infective charge

[1] Assistant Professor of Entomology and Assistant Entomologist in the Experiment Station, Division of Entomology, University of California, Berkeley, California.

[2] Watson, M. A. The transmission of beet mosaic and beet yellows viruses by aphides; a comparative study of a non-persistent and a persistent virus having host plants and vectors in common. Proc. Roy. Soc. Lond., B, 133: 200–219. 1946.

could be built up in an individual, and that the charge would be dissipated during feeding.

In each of the 30 replications, the aphids and test plants were selected at random from a population. A single virus source was used for all series in any one replication, and the order in which a particular series was used within one replication was randomized. A total of 13 different virus sources was used in the experiment, and a single virus source was used for a minimum of one and a maximum of six replications, with a mean of 2.3 replications for each virus source.

RESULTS

Table 1 gives the results in detail of the 30 replications, illustrating the manner in which the infections were distributed and also the frequency with which particular distributions occurred.

The initial infection feeding was to be 30 sec. in duration, but in practice it varied from 20 to 30 sec., with a general mean of 28.40 sec. The range of the initial infection feeding varied from a mean of 27.66 sec. in series H to 29.17 seconds in series A. No significant variation in the infection feeding time could be correlated with the infectivity level, or manner of infection distribution. The mean infection-feeding for the positive results was 28.11 sec., while that for the negative results was 28.58 sec. The same appears to be true in regard to the mean starvation period, as the general mean was 2.22 hr., with a within-series variation of 1.95 hr. in series F to 2.47 hr. in series A. The mean starvation period for the positive results was 2.42 hr., while that for the negative results was 2.08 hr.

The total time required to complete the series, beginning with the initial infection feeding and ending with the last 10-sec. test feeding, had a tendency to decrease from a mean of 16.7 min. in series A to a mean of 11.55 min. in series J, with a general mean of 13.83 min. The total time required to complete the series in the positive results had a mean of 13.98 min.; that for the negative results, a mean of 13.73 min. Since an average of 2.13 min. of the total time used was in actual penetration, there remained an average of 11.7 min. during which the insect was not penetrating plant tissue, resulting in an average of 1.17 min. elapsing between each penetration. These data were collected since the infectivity of aphids transmitting beet-mosaic virus decreases rather sharply with post-infection feeding starvation,[2,3] and it was hoped that some of the results might be explicable upon this basis. However, the data indicate that the effect of this starvation period was fairly uniform in distribution throughout the experiment.

The virus sources used in the replications affected the number of positive plants which resulted in any particular trial. A total of 13 virus sources was used, and the number of positive results in any one replication varied from 0 to 12, where a group of replications was run with a common virus source. The highest number of replications with a single virus source was

[3] Sylvester, E. S. Beet-mosaic virus–green peach aphid relationships. Phytopath. 39: 417–424. 1949.

TABLE 1.—*Distribution of infections[a] obtained when infective single green peach aphid apterae are fed consecutively upon healthy beet seedlings. The insects were previously starved, then given an initial 20–30-second infection feeding. In the progressive series, the infection feedings were increased by one 10-sec. period, while the test plants were decreased by one. The test feeding was 10 sec.*

Series A
Additional infection feedings 0
Successive plant number

1	2	3	4	5	6	7	8	9	10	Frequency[b]
+	–	–	–	–	–	–	–	–	–	3
+	–	+	–	–	+	–	–	–	–	1
+	–	–	+	+	–	–	–	–	–	1
–	+	–	–	–	–	–	–	–	–	1
–	+	+	–	–	–	–	+	–	+	1
–	+	–	–	+	–	–	–	–	–	1
–	–	+	+	–	–	+	–	–	–	1
–	–	–	+	+	–	–	+	–	–	1
–	–	–	–	+	–	–	–	–	–	1
–	–	–	–	–	–	+	–	–	–	1

Series B
Additional infection feedings 1
Successive plant number

1	2	3	4	5	6	7	8	9	Frequency
+	–	–	–	–	–	–	–	–	2
+	+	–	–	–	+	–	–	–	1
+	–	–	–	+	+	–	–	–	1
+	–	–	–	–	–	+	–	+	1
–	+	–	–	–	–	–	–	–	3
–	+	–	–	–	–	–	+	–	1
–	+	–	–	–	–	–	–	+	1
–	–	+	–	–	–	–	–	–	1
–	–	–	+	–	–	–	–	–	1
–	–	–	–	+	–	–	–	–	1
–	–	–	–	–	+	–	–	–	1
–	–	–	–	–	–	–	–	+	1
–	–	–	–	–	–	–	–	+	1

Series C
Additional infection feedings 2
Successive plant number

1	2	3	4	5	6	7	8	Frequency
+	–	–	–	–	–	–	–	1
+	+	+	–	–	–	–	–	1
–	+	–	–	–	–	–	–	2
–	+	–	+	–	–	–	–	1
–	+	–	–	–	–	+	–	1
–	+	–	–	–	–	+	–	1
–	–	+	–	–	–	–	–	1
–	–	+	–	–	–	+	+	1
–	–	–	+	–	–	–	–	1
–	–	–	–	+	–	–	–	1
–	–	–	+	–	–	–	+	1
–	–	–	–	–	+	–	+	1
–	–	–	–	–	–	+	–	1
–	–	–	–	–	–	–	+	1

Series D
Additional infection feedings 3
Successive plant number

1	2	3	4	5	6	7	Frequency
+	–	–	–	–	–	–	2
+	+	–	+	+	–	–	1
+	–	–	+	–	–	–	1
–	+	–	–	–	–	–	1
–	+	+	–	–	–	–	1
–	+	–	–	–	–	+	1
–	–	+	–	–	–	–	3
–	–	–	+	–	–	–	1
–	–	–	–	+	–	+	1
–	–	–	–	–	+	–	1

Series E
Additional infection feedings 4
Successive plant number

1	2	3	4	5	6	Frequency
+	–	–	–	–	–	6
+	+	–	–	–	–	1
+	–	+	–	–	–	1
–	+	–	–	–	–	2
–	+	–	–	–	+	1
–	–	–	–	+	–	2
–	–	–	–	–	+	1

Series F
Additional infection feedings 5
Successive plant number

1	2	3	4	5	Frequency
+	–	–	–	–	2
+	–	+	–	–	1
–	+	–	–	–	3
–	+	–	+	–	1
–	–	+	–	–	4
–	–	–	+	–	2
–	–	–	–	+	1

Series G
Additional infection feedings 6
Successive plant number

1	2	3	4	Frequency
+	–	–	–	2
+	–	+	–	1
+	+	–	+	1
–	+	–	–	1
–	–	+	–	2
–	–	–	+	

Series H
Additional infection feedings 7
Successive plant number

1	2	3	Frequency
+	–	–	2
+	+	–	3
–	+	–	2
–	–	+	3

Series I
Additional infection feedings 8
Successive plant number

1	2	Frequency
+	–	7
+	+	1
–	+	5

Series J
Additional infection feedings 9
Successive plant number

1	Frequency
–	
+	4

[a] The plus sign (+) indicates the production of the disease, and the minus sign (–) shows that no disease resulted. Only the positive results of 30 replicates for each series are included.
[b] Frequency refers to the number of times the distribution occurred.

six, and in this particular case the range of infections per replication varied from 6 to 12. In another case where a single virus source was used for two replications, the range was 6 to 16. Consequently, it was felt that the virus source influenced the number of plants infected, but the extreme variation obtained in some cases indicated that the insect population used was also a source of variation.

The variation in patterns of the distribution of infections (Table 1) illustrates the unpredictable manner in which the insects will discharge the virus. Thus while a given aphid population which has had access to a virus source can be demonstrated to actually contain a certain proportion of infective insects, the particular feeding that will successfully inoculate a host plant cannot be predicted with certainty, except that the probability for successful inoculation tends to decrease as the number of plants fed upon increases.

There is a trend for the insects to dissipate their charge of virus in the first few plants fed upon. Apparently the tendency is that the first two plants fed upon are infected, after which there is a strong tendency for the chances of infection to be in almost a linear relationship, up to and including the tenth plant. The drop between the infections obtained upon the second plant and those obtained upon the third is rather pronounced, but not significant ($\chi^2 = 3.50$ for one degree of freedom).

The summarized results are in table 2. While the total number of infective insects (119 out of 300, or 39.7 per cent) was relatively high, the total number of plants infected (170 out of 1650, or 10.3 per cent) was relatively

TABLE 2.—*Summarization of results obtained in trials made to determine the distribution of infections obtained when single infective green peach aphids are fed consecutively upon healthy beet seedlings. The insects were starved, then given an initial 20–30-sec. infection feeding. In the progressive series (A through J), the infection feedings were increased by one 10-sec. period, while the test feedings, each 10 sec. in duration, were decreased by one*

Series	Number of plants infected	Number of aphids infective	Mean number of plants infected per aphid	Frequency with which a single aphid infected 1, 2, 3, or 4 plants			
				Plants			
				1	2	3	4
A	24/300[a]	12/30[b]	2.00	6	1	4	1
B	25/270	16/30	1.56	10	3	3	0
C	26/240	15/30	1.73	7	5	3	0
D	20/120	13/30	1.53	8	4	0	1
E	17/180	14/30	1.21	11	3	0	0
F	16/150	14/30	1.14	12	2	0	0
G	11/120	8/30	1.97	6	1	1	0
H	13/90	10/30	1.30	7	3	0	
I	14/60	13/30	1.07	12	1		
J	4/30	4/30	1.00	4			
			Total	83	23	11	2

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

[b] In ratios listed, numerator is number of aphids infected; denominator, number of aphids tested.

low, a mean of 1.43 plants being infected per aphid. Between the various series, there was a little variation in the mean number of plants infected per aphid ranging from a mean of 2.0 plants infected per aphid in series A to a mean of 1.00 plants infected per aphid in series J. The general impression given is that the infective potential of the insects cannot be improved to any significant degree by increasing the number of 10-sec. infection feedings, in spite of the fact that 10 sec. is within the acquisition threshold value.[3] On the other hand, there is no appreciable drop in the infectivity potential as long as the 10-sec. feedings are restricted to the virus source, but if the 10-sec. feedings are upon healthy plants there is dissipation of the virus charge.

Approximately 70 per cent of the infective insects, regardless of the number of infection feedings allowed, were capable of infecting only one plant out of the series fed upon, another 19 per cent infected two plants, while only 9 per cent infected three, and 1.6 per cent infected four plants. In no case was there sufficient infective charge to enable an aphid to successfully inoculate more than four plants. These results would seem to indicate that there is some factor, or factors, within the insect's feeding mechanism that not only limits the charge of virus that can be acquired, but also the number of infections that any given insect can produce. While it can be stated with fair accuracy that nearly 90 per cent of the infective population had an infectivity potential that enabled it to inoculate a maximum of two healthy plants, no definite predictions could be made as to the particular plants that would be infected during a series of stylet penetrations, with the exception that chances for infection decreased as the number of plants fed upon increased.

Possibly the initial puncture made upon the virus source was the infectivity determinant, and additional punctures upon the virus source were of no consequence in either increasing or decreasing the virus charge. To test this point another experiment was designed, in which a population of starved green peach aphids was divided into two lots. Half of the individuals were given a 15-sec. infection feeding upon a virus source, and then were transferred to a healthy beet seedling. The other half of the population were allowed a 15-sec. feeding on the healthy plant, then given a 15-sec. infection feeding on the virus source, and then returned to the test plant for the 1-hr. test feeding. Six replications, using five plants per treatment, were made. The insects that fed upon the virus source and then on the healthy test plant inoculated 9 of the 30 plants, while the insects that fed initially on the test plant, then on the virus source, and then again on the test plant inoculated 8 of the 30 plants. Another trial was made, again using six replications and five plants per treatment. In this case the insects which fed upon the virus source were allowed two separate 15-sec. infection feedings, while the other group was used in the same manner as in the previously reported experiment. This was done to equalize the stylet penetrations effected by both groups prior to the final test feeding. In this experiment,

the insects allowed two separate infection feedings upon the virus source inoculated 6 of the 30 plants, while the insects allowed one initial healthy plant feeding, then the infection feeding, inoculated 5 of the 30 test plants. Evidently allowing the starved aphids an initial feeding upon a healthy plant before an infection feeding does not affect the infectivity potential which can be obtained during a 15-sec. infection feeding.

Consequently, while the initial infection feeding, following a period of starvation, can permit the acquisition of near maximum infectivity potential, subsequent infection feedings can be just as effective; but in any case, the subsequent infection feedings can do little to increase the infectivity potential, if that potential has been previously satisfied.

SUMMARY AND CONCLUSIONS

The results of tests conducted on multiple infection feeding and serial transfer to additional host plants are of interest in several respects. Perhaps the most pertinent observation is that increased separate infection feedings for the aphids do not materially influence the level of infectivity. While it has been demonstrated that the insects can increase their efficiency of transmission of the virus by making a series of separate stylet penetrations upon the healthy test plant[3], multiple stylet penetrations upon the virus source plant are of little benefit. Possibly the inactivator hypothesis[4] can be used to explain the failure of either an increase or decrease in virus charge during separate stylet penetrations upon the virus source. The initial charge might have been inactivated, but was renewed during the next penetration, with no change in infectivity level. However, when this reasoning is extended to the results obtained when the insects were allowed to feed initially in healthy plants, difficulties are encountered unless saturation of the inactivator occurs with non-virus feeding. While the inactivation of the virus might suggest the reason for approximately 90 per cent of the infective aphids effecting a maximum of two successful inoculations, there is no obvious reason why the infections produced are distributed in almost a random manner. If the inactivator was produced during feeding, and the charge to be inactivated was approximately the same, then it would be expected that the inactivation would occur during the first or second test feedings, and that this effect could be readily demonstrated.

From the field standpoint, it is encouraging to have experimental indications that the virus-carrying potential of infective aphids is limited in such a way, and that another unknown factor tends to reduce the amount of virus spread that occurs under such conditions of vector activity.

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UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

[4] Watson, M. A., and F. M. Roberts. A comparative study of the transmission of Hyoscyamus virus 3, potato virus Y and cucumber virus 1 by the vectors *Myzus persicae* (Sulz.), *M. circumflexus* (Buckton), and *Macrosiphum gei* (Koch). Proc. Roy. Soc. Lond., B, 127: 543–576. 1939.

TRANSMISSION OF BRASSICA NIGRA VIRUS BY THE GREEN PEACH APHID

EDWARD S. SYLVESTER[1]

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Bawden and Pirie in 1939 reported on the purification of potato virus Y and Hyoscyamus virus 3, both of which are aphid-transmissible, and gave evidence indicating that these viruses were similar in some respects to potato-X virus (2). With the isolation and electron micrography (6) of squash-mosaic virus came the first direct revelation of the size and shape of an aphid-transmissible virus. The squash-mosaic virus particles are spherical, approximately 30 mμ in diameter, and, consequently, indicate that some of the aphid-transmissible viruses are spherical. However, this virus is somewhat anomalous, inasmuch as Freitag (3) has reported it to be transmitted by beetles as well as by aphids.

The isolation, purification, and subsequent electron micrography of the *Brassica nigra* virus by Takahashi (5) gave the second illustration of an aphid-transmissible virus. The long, flexuous rods of the *Brassica nigra* virus resemble the particles of the potato-X virus, but insect transmission of the latter is unknown, in spite of the fact that the virus is highly contagious in nature, is found in relatively high concentrations in the host plant, and is comparatively stable *in vitro*. One explanation for the failure of insects to transmit the potato-X virus is that the particles of such viruses as potato-X virus and tobacco-mosaic virus may be too large to permit free transit through the aphid's food and salivary canals, especially the latter (1).

It was thought advisable to test the aphid transmissibility of *Brassica nigra* virus, since particle size and form of the virus closely resembled those of potato-X virus.

MATERIALS AND METHODS

The original virus source was a diseased plant of *Brassica juncea* Czern. et Cross. obtained from W. N. Takahashi. Subinoculations from this original plant, by mechanical inoculation and by the green peach aphid *Myzus persicae* (Sulzer), furnished additional sources throughout the experimental work. The test plants, seedlings of *Brassica juncea*, a host favored by Takahashi, were inoculated when in the cotyledon stage. The aphids were obtained from stock noninfective cultures and were transferred with a camel's-hair brush. The feeding periods were timed with a stopwatch, and test plants, after being fed upon by the insects, were fumigated with nicotine before being placed in the greenhouse for incubation.

Takahashi repurified the virus from plants which had been inoculated by

[1] Assistant Professor of Entomology and Assistant Entomologist in the Experiment Station, Division of Entomology, University of California, Berkeley, California.

The author is indebted to W. N. Takahashi, who supplied the original source of the virus.

the aphids, in order to confirm the size and shape of the virus. In his opinion all preparations were identical with the original material.[2]

RESULTS

Since work with the nonpersistent beet-mosaic virus and the green peach aphid had indicated (4) that the acquisition threshold was near 10 sec. for that particular vector-virus relationship, the infection feeding intervals used for the determination of the acquisition threshold were 5, 10, 15, 20, 25, and 30 sec. Single noninfective green peach aphid apterae, after being starved in a vial for 1 to 2 hr., were transferred to a virus source plant for the desired infection feeding. After completion of the infection feeding, they were transferred to a healthy seedling of *Brassica juncea* for a test feeding of 1 hr. A total of six replications, five plants per treatment, were made, using a common virus source for each replication.

None of the 30 plants was infected when the infection feeding was restricted to 5 sec. Three of 30 plants were infected after the 10-sec. feeding period; 16 after the 15-sec. feeding; 23 after the 20-sec. feeding; 15 after the 25-sec.; and 8 after the 30-sec. feeding period.

The results are similar to those obtained for the acquisition threshold of the green peach aphid transmitting the beet-mosaic virus (4). The green peach aphid can acquire the *Brassica nigra* virus after feeding upon an infected plant for a period of time ranging between 6 and 10 sec. The gradual rise in efficiency from the 10-sec. period until the peak at 20 sec., then the decline in efficiency during the 25- and 30-sec. intervals have no adequate explanation except possibly by the inactivator hypothesis (7); i.e., during the initial phases of penetration there is no inactivator present to act upon the virus being acquired, the rise in efficiency being due to the insect's gradually reaching the optimum depth or duration of stylet penetration for maximum virus pick-up. The decline in efficiency that follows might be due to the gradual introduction of recently secreted inactivator.

The next phase investigated was the establishment of the inoculation threshold (4). The same time intervals used in the acquisition threshold were employed, but applied to the length of the test feeding period. Single noninfective green peach aphid apterae were starved in a vial for 1 to 2 hr., given a 20-sec. infection feeding, and immediately transferred to the test plant for the desired test feeding interval. Again six replications, five plants per treatment, of each interval were used, with a virus source being at least common to any single replication. Four of 30 plants were infected after the 5-sec. feeding period; one after the 10-sec. feeding; five after the 15-sec. feeding; 13 after the 20-sec. feeding; 7 after the 25-sec. feeding; and 11 after the 30-sec. feeding period.

An infective green peach aphid can successfully inoculate a healthy plant with the *Brassica nigra* virus in as short a time as 5 sec., but if a longer interval of time is used, e.g., 20 sec., a little better efficiency can be expected.

[2] Personal communication.

Once the acquisition and inoculation thresholds were established, a value could be determined for the transmission threshold (4). Fifty green peach aphids, previously starved, were given a 10- to 15-sec. infection feeding upon a diseased source plant and then immediately transferred to healthy seedlings where they were allowed a maximum test feeding of 20 sec. Twelve of the 50 aphids successfully transmitted the virus. The data on the 12 individuals indicated that the mean infection feeding was 14.5 sec., with a range of 11 to 15 sec.; the mean transfer time (the time which elapsed while the insect was moved from the diseased plant to the healthy plant and commenced to feed) was 26.9 sec., with a range of 11 to 55 sec.; and the mean test feeding period was 18 sec., with a range of 13 to 20 sec. Based upon these data, a transfer threshold value was obtained which had a mean value of 59.5 sec., approximately 1 min., with a range of 39 to 90 sec. Thus, as in the case of the beet-mosaic virus, the insects can, under certain conditions, complete the transmission cycle in less than a minute, possibly in 30 seconds.

SUMMARY AND CONCLUSIONS

Replicated tests, using the green peach aphid as the vector and the *Brassica nigra* virus of Takahashi as the virus, have indicated that the insect can acquire an infective charge of virus during 6 to 10 sec. of infection feeding and can inoculate a healthy plant successfully during the first 5 sec. or less of the test feeding period. By employing optimum values for both the acquisition and inoculation thresholds, a transmission threshold value was obtained, with a mean of approximately 1 min. and a minimum of little more than 30 sec.

The significance of the work lies in the fact that the *Brassica nigra* virus is similar in shape and size to potato-X virus, a virus which, to date, has not been transmitted by the green peach aphid or other insect. It appears that the size and shape of the virus particle are not the limiting factors in determining its status in respect to aphid transmissibility.

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UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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BLOSSOM-END ROT OF PEPPER (CAPSICUM ANNUUM L.) IN NEW MEXICO[1]

PHILIP J. LEYENDECKER, JR.[2]

(Accepted for publication February 20, 1950)

Leonian (6) in 1922 described a new species, *Phytophthora capsici* Leon., which caused a stem and fruit blight of chile[3] peppers in New Mexico. According to him the pathogen produced linear, dry lesions of various sizes at or near the blossom ends of the infected fruits. He noted that in the field infected tissues soon dry, bleach to a dull straw color, sink below the level of the healthy tissues, and finally become parchmentlike. A photograph of the field symptoms accompanied the description and is reproduced in figure 1, B. Leonian was puzzled because he could not reproduce these field symptoms under controlled greenhouse conditions. In the greenhouse the infection did not remain localized in small linear areas at the tip end of the fruit, but the entire fruit became mummified and covered with yellow-tan sporangial masses. Leonian attributed this difference in reaction to a "mass action" phenomenon as advanced by Smith (8) and Heald (5).

Recent workers (1, 4, 9) have been unable to confirm the blossom-end field symptoms of *Phytophthora* described by Leonian in New Mexico. In each instance, under ideal conditions for the expression of the disease, the fruits became mummified and completely overgrown by the pathogen. Weber (9), working with the parasite in Florida, was unable to account for the great differences in field symptoms produced in New Mexico and Florida. He expressed the opinion that differences in symptoms might be attributable to dissimilarities in relative humidity in the two States.

During the 1947 and 1948 seasons at the New Mexico Experiment Station farms, the author made additional notes and observations on the disease which seem to clarify the work of Leonian (6). In 1947, a severe epiphytotic of Phytophthora blight (7) made it possible to study in detail field symptoms of the disease on chile pepper. Blight was extremely severe; on many plants the entire crop of green peppers became infected. Within 12 to 24 hr., the diseased fruit became completely covered with yellowish-tan sporangiophores and within another 12 hr. they became dry and mummified (Fig. 1, C). A pepper plant killed by *Phytophthora* and bearing numerous mummified fruits is shown in figure 1, D.

Workers in Colorado, Georgia, and Florida (1, 4, 9) have described similar field symptoms on bell and chile peppers. Leonian produced such

[1] Paper No. 58, New Mexico Agricultural Experiment Station, State College, New Mexico.

[2] Associate Plant Pathologist.

[3] College Number 9, a hot type chile developed by the New Mexico Experiment Station.

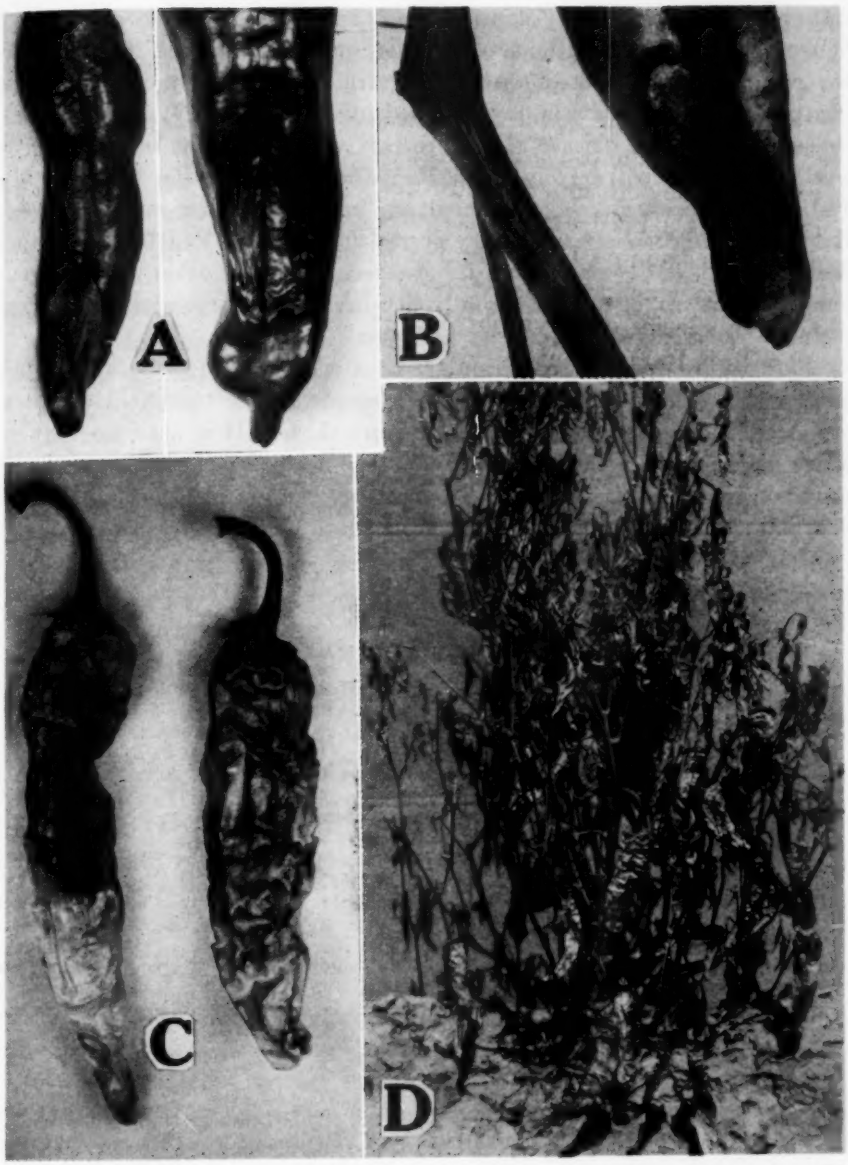

FIG. 1. Blossom-end rot (A), which is similar to the lesions attributed to Phytophthora (B) by Leonian; and Phytophthora blight (C and D) of chile pepper (*Capsicum annuum* L.). B, after Leonian (6).

symptoms in the greenhouse but was unable to produce them artificially in the field.

Early each season during 1947, 1948, and 1949 it was noted that many chile peppers (Fig. 1, B) had symptoms identical to those described by Leonian as Phytophthora blight. The affected area, which was always at the tip end of the fruit, first appeared as a small water-soaked spot which

enlarged, became sunken, straw-colored, and parchmentlike as shown in figure 1, A. Many isolations were made but no pathogenic fungi were recovered from the infected tissue taken from the tip of the fruit. These identical symptoms have been described by Higgins (2, 3) as blossom-end rot, a physiological disease of pepper. It was noted that blossom-end rot was most prevalent early in the season when transpiration rates are high and the root system of the transplants has not become well established. Early-maturing varieties are very susceptible: from 95 to 100 per cent of the early pods had symptoms of the disease. When weather conditions are favorable the infected areas usually are invaded by secondary organisms, the most prevalent being *Alternaria tenuis*.

It therefore appears that Leonian described the field symptoms of blossom-end rot for those of *Phytophthora capsici*. The true field symptoms produced by *P. capsici* are shown in figure 1, C. It is believed that evidence has been presented which clarifies the disagreement of Leonian's observation in New Mexico and those made by workers in Colorado, Florida, Georgia, and other States where Phytophthora blight is present.

NEW MEXICO AGRICULTURAL EXPERIMENT STATION
STATE COLLEGE, NEW MEXICO

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VARIABILITY IN ONION NECK-ROT FUNGI[1]

J. H. Owen, J. C. Walker, and M. A. Stahmann

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In 1917 Munn (5) described a neck-rot disease of bulbs of onion (*Allium cepa* L.) in New York and named the pathogen *Botrytis allii* Munn. In 1925 Walker (10) described a second neck-rot disease of onion in Wisconsin and named the pathogen *Botrytis byssoidea* J. C. Walker. The latter investigator (11) suggested that the disease incited by *B. allii* be designated as gray-mold neck rot and the second disease as mycelial neck rot. He compared the two fungi on the host and on culture media (11). On the host the two were not readily distinguished in the early stages of disease development. Under moist conditions *B. byssoidea* produced an abundance of superficial white mycelium which sporulated sparsely in the early stages of the disease, while *B. allii* produced relatively sparse extramatrical mycelium and a low, dense, gray growth of the conidiophores and conidia. The conidia of the two species were similar in color, shape, and mode of germination but they differed slightly in size. The range of *B. allii* was $4–8 \times 6–16$ μ with most spores falling within the limits of $5–6 \times 8–10$ μ. The range of *B. byssoidea* was $5–11 \times 8–20$ μ, with most of the spores falling within the limits of $7–8 \times 10–14$ μ. On culture media, *B. allii* produced relatively little aerial mycelium, a dense gray mass of conidiophores and conidia covering the surface promptly. *B. byssoidea* formed a profuse quantity of white, fluffy aerial mycelium and sporulation was very rare. On host tissue in advanced stages of decay, both species produced sclerotia which were indistinguishable. The two species were found to produce sclerotia only rarely on potato-dextrose agar and the other culture media used.

Hansen and Smith (3) grew different multinucleate strains of *Botrytis cinerea* Pers. in mixed culture. The progeny of this mixed culture was analyzed by a single-spore method for three generations and resulted in homotypes for both parents and a number of heterotypic strains. The heterotypic strains gave rise to both homotypes and heterotypes. Since hyphal fusion was observed commonly in all strains of this species, they suggested that some of the nuclei of the two mated strains interchanged. As a result a heterocaryotic mycelium resulted which in production of conidia provided a possibility for spores containing only nuclei derived from one or the other parent (homotypic) and for spores containing nuclei of both types (heterotypic). Hansen (2) later showed that in many

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members of the Fungi Imperfecti and Ascomycetes there is a tendency for sporulating lines (S) to throw variants which are mycelial (M) and variants which are heterotypic for M and S. Since this latter phenomenon is now known to occur in species with uninucleate mycelium, it is logical to consider that the variants may arise as the result of mutation. In fact Keitt et al. (4) have shown in a mycelial mutant of *Venturia inaequalis* (Cke.) Wint. that the character is controlled by a single gene pair. It is reasonable to assume, therefore, that mycelial variants may be the result of point mutations.

In view of the close similarity of *B. allii* and *B. byssoidea*, except for sporulation on culture media and a relatively small difference in the range of length and width of conidia, the question which became the basis of this investigation was whether or not one of these species arose as a mutation from the other. Therefore, a study of natural and induced variants in each species was made.

VARIATION IN THE TYPICAL WILD TYPE OF BOTRYTIS ALLII

The type of *B. allii* practically always found in nature is referred to herein as the typical wild type. It was found to be extremely stable when grown on culture media where it constantly produced low, dense mats of heavily sporulating mycelium. It was shown that temporarily nonsporulating cultures could be obtained by transferring hyphae from mycelial tufts which were sometimes found in old cultures (Fig. 1). However,

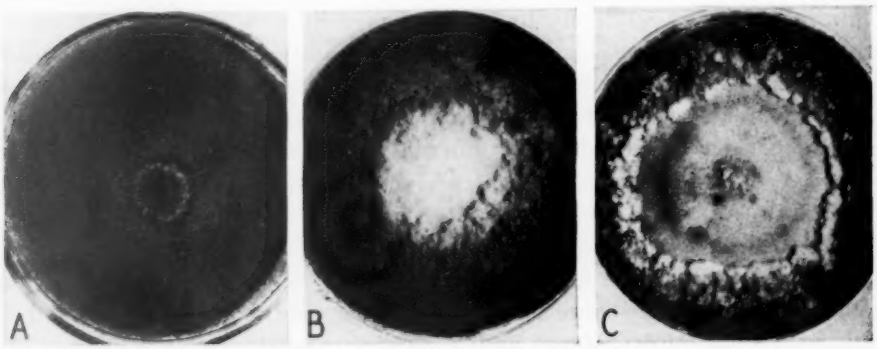

FIG. 1. Variants observed in *Botrytis allii* following hyphal transfers from nonsporulating mycelial tufts. A. Typical wild type. B. Culture with nonsporulating tufts. C. Culture resulting from a hyphal transfer from a mycelial tuft in B.

these nonsporulating cultures promptly reverted to the sporulating wild type when transferred. Further stability was illustrated when numerous single-spore isolations invariably produced typical sporulating cultures.

Various types of culture media had little or no effect on the ability of the fungus to sporulate, but growth was retarded on certain media low in carbon and nitrogen sources. This was in contrast to the results of Newton and Hastings (6), who were able to induce variation in conidium development of *Botrytis tulipae* (Lib.) Lindau by using different carbon

sources in the medium. On media containing high sugar concentrations, the width of the conidia of *B. allii* was atypical. During the younger stages of development the spores produced on all concentrations of sugar were identical. However, as the spores on the media having higher concentrations continued to grow, they became abnormal in width without increasing in length. The conidia were obovate to globose rather than the usual elliptical to pyriform shape. The pronounced increase in spore width may have resulted from greater utilization of carbohydrate. Sclerotia were not observed on culture media.

In general the amount of variability produced by the sporulating wild type was very small, and when variations did occur they usually reverted rather promptly to the wild type.

ATYPICAL WILD FORM OF BOTRYTIS ALLII

A variable form of *B. allii,* found in nature only once, is referred to herein as the atypical wild type. It was isolated from an onion bulb which had been grown in 1947 near Racine, Wisconsin, and had been stored at 40° F. for 10 months. The upper half of the bulb was completely covered with conidiophores, and black sclerotia were numerous around the neck. Tissue transfers were made to potato-dextrose agar, and the resulting cultures gave rise to dense tufts of grayish-white mycelium with scattered groups of conidiophores, quite in contrast to the usual tissue cultures from bulbs which yielded highly sporulating cultures. Fifty single-spore isolations were made from the culture with most sporulation, and these were incubated at the summer temperature of the laboratory room (23° to 32° C.). Although a majority of the single-spore cultures resembled the mother culture, a number of distinct variants occurred. They were placed in nine groups designated, respectively, as *As, A2, A3, A4, A5, A6, A7, A8,* and *A10.* One hundred spores from each variant were measured. Although there was some variation in the size of the spores produced by individual variants, the average spore size was within the range typical of *B. allii* and in no case did it conform to that of *B. byssoidea.*

In order to study variability further, a large number of single-spore isolations was made from each of the variants. After 10 days at 23° to 27° C. the cultures were examined and separated into type classes. They were then placed at 20° C. in order to make a study of sclerotium formation. The typical wild type, the original atypical wild-type isolate, and some of the outstanding characters of the variants are shown in figures 2 and 3. A full description of the variants is presented elsewhere (6).

In the first and second single-spore generations there was a wide range of cultural types. These consisted of heavy sporulation to few or no conidia; various degrees of appressorium development; lack of abundance of sclerotia; variation in size, shape, and distribution of sclerotia. Co-

nidia, when observed and measured, fell within the known range of *B. allii*. Although the variants of the second single-spore generation varied widely in cultural characteristics, it was of interest to note that in the third single-spore generation the amount of variation was greatly reduced. All of the second single-spore generation variants, except *A4*, appeared to follow a definite pattern, and a certain percentage of them was found to

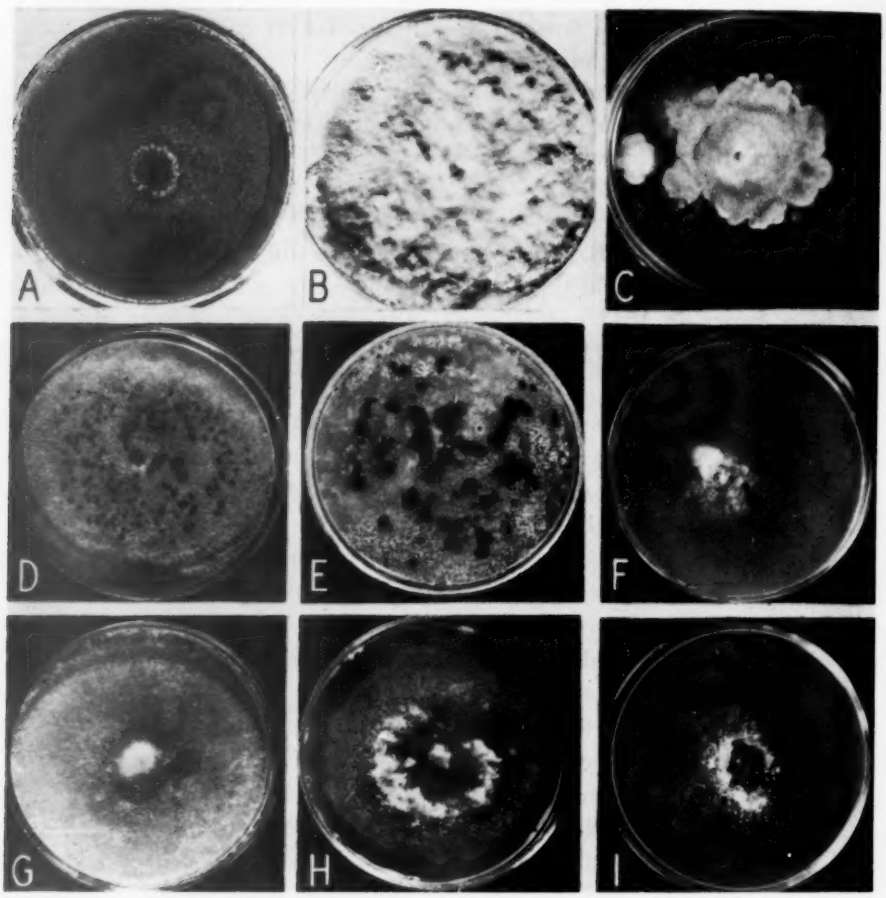

FIG. 2. A. Typical wild type of *Botrytis allii*. B. Original isolate of atypical strain of *B. allii* from which variants shown in this figure and in figure 3 were derived. C. *As* at 14 days. D. *As* at 20 days. E. Single-spore culture from *As* closely resembling latter. F. Single-spore culture from *As* which grew very slowly. G. *A2* at 14 days. H. *A3* at 30 days. I. Single-spore culture from *A3* at 20 days.

be similar to the original variant. *A7* was the most stable, and 34 out of 37 of the third single-spore generation cultures retained the characteristics of *A7*. The appearance of cultures similar to the variant group in the second single-spore generation did not necessarily indicate these cultures to be homotypes. This was illustrated in *A5*, in which in the fourth single-spore generation only 6 per cent of the cultures closely resembled

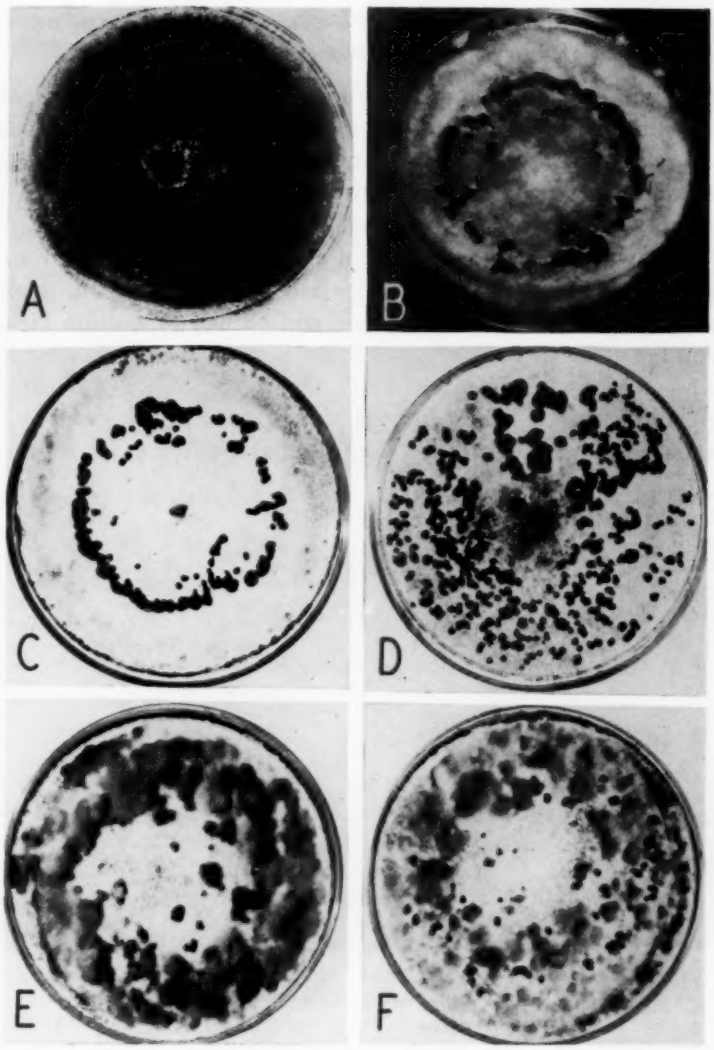

FIG. 3. Variants from atypical strain of *Botrytis allii* (see figure 2). A. *A5*, which is similar to the typical wild type of *B. allii*, at 20 days. B. *A8* at 20 days, viewed from upper side of plate. C. Same as B, viewed from lower side of plate. D. Mass transfer from culture in C, at 20 days. E. *A10* at 20 days. F. Single-spore culture from *A10* at 20 days.

the original variant. Variant groups possessing sclerotia invariably produced a large number of cultures with sclerotia in the third generation.

Representative cultures from several distinct variants were inoculated into onion bulbs. They included cultures with heavy appressorium development and sclerotia, heavy appressorium development and no sclerotia, abundant sporulation and meager appressorium formation, and heavy mycelial growth with few conidia. All cultures were pathogenic and sporulation was commonly abundant on bulbs infected by each.

VARIABILITY IN BOTRYTIS ALLII INDUCED BY A NITROGEN MUSTARD METHYL-BIS (β-CHLOROETHYL) AMINE

Typical wild type. Ten-ml. aliquots of a spore suspension containing 250,000 conidia per ml. were pipetted into two small flasks; one contained 10 ml. of a 0.01 mM. per ml. concentration and the other 10 ml. of a 0.02 mM. per ml. concentration of the nitrogen mustard in a solution buffered with sodium bicarbonate (7). Samples of conidia in the treating solution were withdrawn at intervals chosen in geometric progression from 1 to 64 min. and at 360 min. These were pipetted into 100 ml. of a decontamination fluid containing 60 mg. of glycine and 68 mg. of sodium bicarbonate. After storage at 5° C. for 10 to 12 hr., 1-ml. samples were spread over thin layers of potato-dextrose agar in Petri dishes which were incubated at 24° C. for 15 to 45 hr. When germination had begun, conidia or hyphal tips were transferred to test tubes. After 12 days, cultures from treated and

TABLE 1.—*Variants induced in the typical wild type of Botrytis allii by nitrogen mustard (methyl-bis (β-chloroethyl) amine)*

Treatment (minutes)	Surviving spores		Percentage of variants among survivors[b]
	Number[a]	Per cent	
	0.01 mM. per ml. concentration		
0	237,000	100.00	0.0
1	140,000	59.07	3.6
2	126,000	53.16	5.9
4	24,500	10.34	10.7
8	24,900	10.51	12.8
16	19,000	8.02	10.0
32	14,750	6.22	8.8
64	9,400	3.97	2.7
360	3,880	1.64	0.0
	(A) *0.02 mM. per ml. concentration*		
0	237,000	100.00	0.0
1	29,550	12.47	0.0
2	26,250	11.08	0.0
4	10,000	4.22	3.1
8	3,750	1.58	27.3
16	3,450	1.46	30.6
32	1,475	0.62	44.2
64	600	0.25	74.5
360	20	0.0008	12.5
	(B) *0.02 mM. per ml. concentration used on survivors in A*		
0	6,000	100.00	0.0
1	3,500	58.33	0.0
2	3,750	62.50	0.0
4	3,800	63.33	0.0
8	2,500	41.67	0.0
16	2,500	41.67	2.0
32	1,750	29.17	2.0
64	2,150	35.83	2.0

[a] Values given are calculated by multiplying the average number of spores surviving in each of four plates by the amount of dilution each sample received.

[b] Calculated from a random sample of 100 single spores from the surviving colonies of the treatments except, in the 32-, 64-, and 360-min. treatments in (A), in which case all of the survivors were saved.

from untreated conidia were compared and the variants were transferred.

The 0.02 mM. per ml. concentration (Table 1) killed a larger percentage of the conidia than the 0.01 mM. per ml. concentration, and an increase in percentage of variants from the 4-min. to the 64-min. treatment was followed by a marked decrease at the 360-min. treatment. Nearly 75 per cent of the cultures from the 64-min. treatment differed from the control in cultural characteristics.

The variability induced covered a wide range of cultural characteristics, but many of the variants were quite similar. Most of them were mycelial in character as in figure 4. The mycelial cultures were whitish

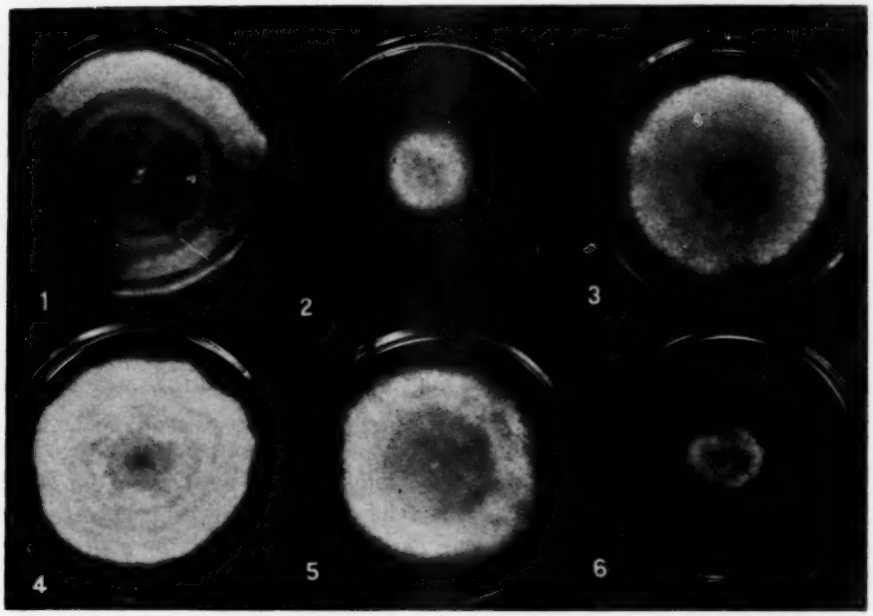

FIG. 4. Five representative 8-day-old variants of *Botrytis allii* induced by nitrogen-mustard treatments, compared with the untreated culture (1). Cultures 2, 4, and 5 are nonsporulating.

to gray and became darker in the center as they aged. Some were mycelial for only a few days, when a scanty production of conidiophores occurred along the periphery of the cultures. All others began to sporulate after several successive mass transfers. Nearly all of the variants grew more slowly than the untreated culture; others showed various degrees of staling and of production of yellow-to-brown pigment. Some variants produced only one or two hyphal strands which grew to a length of 3 to 6 mm. Mere tufts of mycelium 2 to 3 mm. in diameter were often produced, and the number of conidiophores on these tufts varied.

Eight of the mycelial variants were selected for further study. After the first transfer, five of the variants developed a few conidiophores around the periphery as they aged. Three cultures which remained my-

celial for 4 to 5 successive mass transfers and then produced increasing amounts of conidiophores with further transferring were highly pathogenic. Conidiophores were produced normally on the onions, and the conidia were found to correspond with those of *B. allii* in color, size, and shape.

As soon as one of the mycelial cultures sporulated, its conidia were treated with a 0.02 mM. per ml. concentration of nitrogen mustard. The spore kill as presented in table 1, B, was very slight, indicating that the conidia of survivors of the first treatment had a much higher resistance to the nitrogen mustard than those of the parent culture. All of the cultures except three were identical with the cultures of the untreated conidia of the variant. These three new variants were mycelial at first, but conidiophores occurred after the first transfer.

Atypical wild type. A single-spore culture from *A5* which appeared to be identical with the original *A5* variant was selected. This variant produced an abundance of conidia and an appressorial band 1 to 2 mm. in width on the side of the glass tube, but did not form sclerotia. It was, in fact, among all of the atypical culture variants, the one most similar to the typical wild type. A heavy spore suspension of the variant was treated with the nitrogen mustard in the same manner as was the typical wild type.

Of 100 untreated spores, 98 resulted in macroscopically identical cultures which varied from the mother culture only in that conidia were most abundant at the top of the slant. One spore yielded a culture which staled at an early stage, forming a small tuft of mycelium.

When 10 ml. from the same spore suspension was treated with a 0.01 mM. per ml. concentration, the spore kill was so slight that the entire treatment was discarded. Even the 0.03 mM. per ml. concentration of the nitrogen mustard was not strong enough to kill all of the spores at 64 min., although in previous treatments all spores of other strains of *B. allii* were killed with this concentration. It appeared, therefore, that this variant of the atypical wild type was more resistant to nitrogen mustard than was the typical wild type.

Nitrogen mustard had a definite effect on the period of germination of the spores. The untreated spores began to germinate at 14 to 20 hr., but 60 hr. were required for the treated spores. The germination period became longer as the length of the treatment increased. As shown in table 2, the percentage of variants from the treated spores increased as the time of treatment was lengthened, until the maximum was reached at 16 min. Treatments longer than 16 min. resulted in a gradual decrease in the number of variants.

The variants had wide differences in cultural characteristics, and the same type of variant was found to appear frequently in different treatments. Although the majority of the cultures grew rather rapidly, many of them showed different degrees of staling. A few merely developed

TABLE 2.—*Variation induced in the A5 line of Botrytis allii by nitrogen mustard (methyl-bis (β-chloroethyl) amine) at 0.03 mM. per ml.*

Treatment (minutes)	Surviving spores		Percentage of variants among survivors[b]
	Number[a]	Per cent	
0	136,000	100.00	2.0
1	90,000	66.18	12.1
2	37,500	27.57	33.3
4	24,350	17.90	28.0
8	8,200	6.03	32.5
16	1,700	1.25	45.2
32	700	0.51	23.0
64	100	0.07	16.6

[a] Values given are calculated by multiplying the average number of spores surviving in each of four plates by the amount of dilution each sample received.

[b] Calculated from a random sample of 100 single spores from the surviving colonies of the no-treatment through the 8-min. treatment; from the rest of the treatments all of the survivors were saved.

several hyphal strands 3 to 4 mm. in length; in others, small aerial tufts 3 to 5 mm. in diameter were formed. In some variants appressorium production was extremely heavy, consisting of a black band 6 to 8 mm. wide on the glass side of the tube. Other cultures produced bands 1 to 2 mm. in width or hardly visible in some instances. The number of conidiophores produced varied from those cultures in which they covered the substrate to those in which there were only a few scattered groups. In some of the cultures conidiophores were formed only on the appressoria, while in others they appeared most abundantly around the periphery of the culture, within the appressorial band. In most of the variants the mycelium was whitish with few or no aerial tufts. Sometimes white tufts on the appressorial band bore numerous conidiophores. The mycelium of many of the cultures produced a pigment which ranged from light orange to dark brown.

After the cultures had been examined for variants, they were incubated at 20° C. for 2 weeks. When they were reexamined, 71 per cent of the nontreated cultures had developed sclerotia, and 6 per cent had increased their conidium production until they were identical with the original *A5*. Sixty to 70 per cent of the treated cultures from each treatment produced sclerotia, while those which were mycelial or pigmented usually produced no sclerotia. In some there was a slight increase in the production of conidiophores.

Although the range in variability produced by nitrogen mustard in the atypical and typical wild forms was similar, the frequency of mutations induced in the survivors was greater in the atypical form. The atypical form was also much more resistant to the nitrogen mustard than was the typical wild form.

NATURAL VARIATION IN THE WILD TYPE BOTRYTIS BYSSOIDEA

Walker (10) found *B. byssoidea* to be very stable on culture media.

He reported that the fungus produced a rank-growing, fluffy, white mycelium with seldom, if ever, any indication of sporulation. However, variability occurred in two of his cultures when they were exposed to out-of-door winter conditions for 2 to 3 months at Madison, Wisconsin.

Single-spore and mycelial selection. In many genera of the Fungi Imperfecti there is a tendency for the cultures to become mycelial unless frequent single-spore transfers are made from sporulating cultures (2). In order to study the effect of repeated single-spore transfers of *B. byssoidea*, it was necessary to obtain spores from diseased onion bulbs. Sixty-two single-spore isolations were transferred to potato-dextrose agar, and in each case, a nonsporulating mycelial culture was produced. Mycelium from some of the isolates was used to reinoculate onion bulbs which were placed in glassine bags and incubated at 20° C. Ten of 12 inoculated bulbs became infected. Disease development was rapid and sporulation was profuse. A spore suspension was made from the bulb on which was the most sporulation, and 50 single-spore isolations were again transferred to potato-dextrose agar. These second-generation spores likewise produced nonsporulating cultures on the culture medium. Although onions inoculated with these cultures bore larger quantities of spores than were produced with the first two inoculations, no variants that sporulated on potato-dextrose agar were secured.

Relation of temperature to sclerotium formation. Although sclerotia of *B. byssoidea* develop regularly on infected onions, their formation on culture media at room temperature is very uncommon. Walker (11) induced sclerotia in one of his cultures by placing young tube cultures out-of-doors, during midwinter, for 2 to 3 months at Madison, Wisconsin. The same experiment with another culture of *B. byssoidea* failed to induce development of sclerotia. In this investigation transfers of mycelium to potato-dextrose agar in Petri dishes were incubated at room temperature (22° to 25° C.) for 4 days. Four dishes were then placed at each of the following temperatures: 4°, 8°, 10°, 12°, 16°, 20°, 24°, and 28° C. Sclerotial bodies first began to appear at 8° and 10° C. after 10 days of incubation. In these cultures the mycelium had rounded up into numerous compact clumps, and in several instances the distinct outline of the sclerotium could be detected. After 21 days the sclerotial bodies had become more numerous and were mostly white to cream-colored. At 4°, 8°, and 10° C. a few of the sclerotial bodies were beginning to turn black, while at 12° C. the fluffy, white, aerial mycelium had just begun to round up into compact balls. After 33 days approximately one-half of the sclerotia had turned black at the lower temperatures (4° to 8° C.), numerous young sclerotia had appeared at 12° C., and three sclerotiumlike bodies were noticed in one of the cultures at 16° C. for the first time. The most favorable temperature for the formation and the development of sclerotia was 8° C., and all cultures held at this temperature formed from 25 to 35

sclerotia within 45 days. The upper limit for sclerotium formation was apparently 16° C. and the total number of sclerotia produced in the four cultures at this temperature was less than 10. The main changes occurring in the cultures after 33 days were the increase in number and maturity of the sclerotia and a general shrinking of the mycelium until it no longer appeared fluffy but lay flat on the surface of the medium. The sclerotia appeared as balls of compact hyphae, which soon developed into cream-colored sclerotia, gradually turning black. They averaged from 2 to 5 mm. in diameter, 2 to 3 mm. in height, and one-half to 1 mm. in thickness. The shape was irregular, the upper surface was concavely rounded, and the lower surface was convex. In most cases the sclerotia were slightly embedded in the medium, but sometimes they were completely submerged. The mycelium retained its natural white color throughout the entire experiment at temperatures below 20° C., whereas that in the cultures held at 20° C. or above gradually took on a light brownish color which became darker with age.

Relation of medium to growth and sporulation of Botrytis byssoidea. A wide range of culture media was used in an attempt to induce sporulation of *B. byssoidea.* Various vegetable decoctions with dextrose or peptone or both were studied. The production of mycelium was usually profuse, but no sporulation occurred. Czapek's medium with dextrose or peptone or both gave similar results. In one instance conidia were produced on onion tissue planted on an agar plate, but no spores were formed on the mycelium growing on the agar. In a synthetic medium[2] the 20 gm. of dextrose was replaced by sodium potassium tartrate (10 gm.), sodium citrate (10 gm.), sodium acetate (10 gm.), and potassium oxalate (10 gm.), respectively. The presence of dextrose as a carbon source resulted in a fast-growing mycelium and evidently was responsible for sclerotium development at temperatures of 4° to 12° C. When sodium potassium tartrate, sodium citrate, sodium acetate, or potassium oxalate was substituted for dextrose as a carbon source there was significantly less mycelial growth and no sclerotium formation, and with sodium citrate and potassium oxalate there was production of conidia. Sodium potassium nitrate and sodium acetate were more favorable for mycelium development than either sodium citrate or potassium oxalate. The optimum temperature for growth of mycelium was 20° C. with all of the media except potassium oxalate and sodium potassium tartrate, on which the optimum growth occurred at 24° C. Sodium citrate and potassium oxalate induced a scanty growth of mycelium which was hardly visible with the naked eye until the cultures were held up to light. With these two carbon sources conidia were produced on the scattered hyphae. Sodium citrate induced sporulation at 12°, 16°, and 20° C. in 24 days, 20° C. being

[2] $(NH_4)_2SO_4$, 2 gm.; K_2HPO_4, 0.5 gm.; $MgSO_4$, 0.2 gm.; NaCl, 0.2 gm.; dextrose, 20 gm.; agar, 15 gm.; water, 1 L.; pH 6.8.

the most favorable for production of conidia. Potassium oxalate induced a few conidia at 20° and 24° C. in 24 days.

Effect of uranium nitrate on formation of sclerotia and growth of Botrytis byssoidea. Variation in certain fungi induced by uranium nitrate has been reported (9). This chemical was added to potato-dextrose agar at rates of 0.25, 0.50, and 0.75 per cent, and the pH of the medium was corrected to pH 6.8 with N/1 NaOH. Mycelial transfers were made from an 8-day culture of *B. byssoidea* to 20 Petri dishes of each uranium nitrate concentration and to 20 control plates of potato-dextrose agar. These cultures were then incubated at 24° C. and examined at 3-day intervals. The presence of the uranium nitrate in the medium produced noticeable changes in the growth of the cultures as compared with the control cultures. During the first 3 days, growth at all three concentrations consisted of white fluffy mycelium in colonies from 14 to 23 mm. in diameter. After 6 days the characteristics of the mycelium remained unchanged at the 0.25 and 0.50 per cent concentrations, but produced white aerial tufts around the periphery of the cultures at the 0.75 per cent concentration. The control cultures grew twice as rapidly as those at the 0.25 per cent concentration and at three times the rate of those at the 0.5 and 0.75 per cent concentrations.

Several black sclerotiumlike bodies, 3 to 4 mm. in diameter, had appeared in cultures of all concentrations after 9 days of incubation. In the control cultures the mycelium was fluffy and white, and there were no signs of sclerotia. Numerous rings of sclerotia had been produced in the cultures on uranium nitrate medium when they were examined after 12 days of growth. Greatest sclerotium formation took place on the 0.25 per cent uranium nitrate medium, and with further increase in concentration there was a decrease in sclerotium formation.

A yellow-to-orange pigment was produced by the fungus after 12 days of growth. This pigment became noticeably darker at the higher concentrations of the uranium nitrate as the cultures aged. The cultures staled as they grew older, and in no instance did the mycelium reach the sides of the Petri dish. When the cultures were 20 days old, new mycelial tufts began to appear around the periphery of the cultures on media with 0.25 and 0.50 per cent uranium nitrate. The cultures at the 0.75 per cent concentration had produced no aerial tufts at this date and had ceased to grow.

Sclerotia were transferred to media containing the same concentration of uranium nitrate and also to control plates of potato-dextrose agar. The cultures growing on the uranium nitrate medium for the second time staled much faster than under the first treatment. However, in nearly every case sclerotia appeared, usually in rings. Where transfers had been made from uranium nitrate medium to plain potato-dextrose agar, there was a return to normal fast-growing, fluffy mycelium, and only an

occasional sclerotium appeared. When such sclerotia were transferred to potato-dextrose agar, sclerotia failed to appear in the resultant culture at 24° C. It appears that the effect of the uranium nitrate was a temporary one.

VARIABILITY IN BOTRYTIS BYSSOIDEA INDUCED BY NITROGEN MUSTARD

Conidia of *B. byssoidea* were obtained from artifically infected onions and were treated with the same nitrogen mustard as was used with *B. allii*. The treated conidia were plated out on potato-dextrose agar, and the surviving conidia were transferred to test tubes. After 12 days of incubation, the percentage of variants was determined by comparison of the cultures produced by treated conidia with those from untreated conidia.

In the first treatment of conidia 0.01 and 0.02 mM. per ml. concentrations of the nitrogen mustard were used (Table 3). Only 5 macroscopically

TABLE 3.—*Variants in Botrytis byssoidea induced by treatment of conidia with the nitrogen mustard (methyl-bis (β-chloroethyl) amine)*

Treatment (minutes)	Surviving spores		Percentage of variants among survivors[b]
	Number[a]	Per cent	
	0.01 mM. per ml. concentration		
0	8,000	100.00	0.0
1	1,250	15.63	0.0
2	1,150	14.38	0.0
4	1,300	16.25	0.0
8	1,270	15.88	0.0
16	800	10.00	0.0
32	600	7.50	0.0
64	350	4.38	0.0
	0.02 mM. per ml. concentration		
0	8,000	100.00	0.0
1	1,250	15.63	0.0
2	750	9.38	0.0
4	100	11.25	0.5
8	300	3.75	0.0
16	50	0.63	0.0
32	75	0.94	0.0
64	25	0.31	0.0
	0.03 mM. per ml. concentration		
0	99,000	100.00	0.00
1	85,000	86.36	4.38
2	58,500	59.09	7.69
4	23,600	23.84	41.06
8	260	0.27	38.97
16	112	0.11	40.00
32	19	0.02	0.00
64	6	0.0006	0.00
128	0	0.00	0.00

[a] Values given are calculated by multiplying the average number of spores surviving in each of four plates by the amount of dilution each sample received.

[b] Calculated from a random sample of 100 single spores from the surviving colonies of the no-treatment in the 0.01 and 0.02 mM. per ml. concentration, and of the no-treatment through the 4-min. treatment in the 0.03 mM. per ml. concentration; from the rest of the treatments all of the survivors were saved.

different variants were produced, and these appeared in the 4-min. treatment of the 0.02 mM. per ml. concentration. The 5 variants were retarded in growth and produced thinly scattered strands of hyphae. Additional variation was shown to be present when random cultures produced by the treated conidia were selected and used to inoculate onions. Conidia produced by the different cultures on these diseased onions varied in size, and an average of 50 conidia from each culture ranged from 6.7 μ to 8.1 μ in width by 9.6 μ to 15.3 μ in length. Many of these cultures were nonpathogenic.

A heavier suspension of conidia was treated with a 0.03 mM. per ml. concentration of nitrogen mustard, and the spore kill was much greater at this concentration. There was a gradual increase in kill from the 1-min. through the 4-min. treatment, but additional increase in treatment time resulted in very extensive killing of spores, all being killed at 128 min. A large increase in percentage of variants was induced in the range of 1 min. to 4 min. From 4 min. to 16 min. the percentage of variants leveled off, and then dropped sharply to no variants at 32 min.

The variants occurring most frequently were slow-growing, staled cultures (Fig. 5), very similar to those mutants of *B. allii* induced by nitrogen

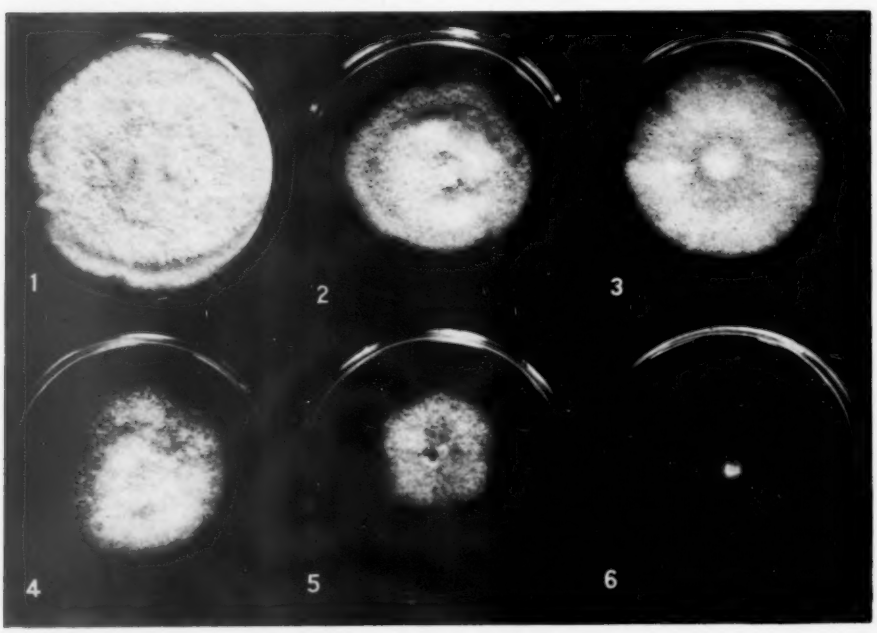

FIG. 5. Five representative variants of *Botrytis byssoidea* induced by nitrogen mustard, compared with the untreated culture (1).

mustard (Fig. 4). The majority of them were merely raised tufts of mycelium, 2 to 5 mm. in diameter. Often only two to four hyphal strands were formed which grew out for a few millimeters. A mass transfer from these staled cultures resulted in the same type of growth. As in the case

of the variants of *B. allii*, those of *B. byssoidea* sometimes grew rapidly for 4 to 6 days accompanied by the production of a light yellow to brown pigment in the medium, and then they ceased to grow further. Numerous flat, black sclerotia 1 to 2 mm. in diameter were found in two of the cultures. Transfers of sclerotia from these two variants produced cultures with white aerial mycelium and no sclerotia. Out of the many variations induced, not one variant was found that produced conidia.

Hyphal cells of *B. byssoidea* were also treated with nitrogen mustard. The rank-growing, fluffy, white aerial mycelium of 7-day-old cultures was scraped into a sterilized Waring Blendor and was cut into fragments 1 to 3 cells in length. Of two concentrations of nitrogen mustard used to treat the hyphal cells, 0.005 and 0.01 mM. per ml. (Table 4), the latter produced

TABLE 4.—*Variants in Botrytis byssoidea induced by treatment of hyphal cells with nitrogen mustard (methyl-bis (β-chloroethyl) amine)*

Treatment (minutes)	Surviving hyphal fragments		Percentage of variants among survivors[b]
	Number[a]	Per cent	
	0.005 mM. per ml. concentration		
0	17,500	100.00	0.00
1	5,500	31.43	11.11
2	5,700	32.57	5.00
4	2,500	14.29	0.00
8	4,400	25.14	7.69
16	2,750	15.71	14.29
32	1,450	8.29	14.89
64	1,450	8.29	0.00
	0.01 mM. per ml. concentration		
0	17,500	100.00	0.00
1	1,500	8.57	15.38
2	1,700	9.71	12.50
4	2,800	16.00	5.88
8	2,500	12.29	45.00
16	1,150	6.57	41.18
32	1,375	7.86	3.57
64	625	3.57	16.70

[a] Values given are calculated by multiplying the average number of fragments surviving in each of four plates by the amount of dilution each sample received.

[b] Calculated from a random sample of 100 single fragments from the surviving colonies of the no-treatment through the 16-min. treatment in the 0.005 mM. per ml. concentration, and of the no-treatment in the 0.01 mM. per ml. concentration; from the rest of the treatments all of the survivors were saved.

the greater kill of the cells and the higher percentage of variants. The irregularity in the percentage of surviving hyphal cells can be explained by the facts that there was a wide variation in the number of cells per fragment and that many of the macerated cells might have been killed before treating. The suspension, however, was placed on an electric shaker during treatment, and the hyphal cells should have been equally distributed throughout the suspension. No sporulation was observed, and the variants induced were very similar to those formed by the treated conidia.

EFFECT OF ULTRAVIOLET IRRADIATION ON BOTRYTIS BYSSOIDEA

Conidia of *B. byssoidea* were suspended in sterile distilled water containing 0.01 per cent of tween 60, an ester of stearic acid used as a detergent. One-half ml. of the spore suspension was pipetted into a Petri dish on a thin layer of potato-dextrose agar and spread over the surface of the medium by tilting. The plates were exposed to ultraviolet radiation for various time intervals (Table 5). An open-type mercury-quartz lamp

TABLE 5.—*Effect of ultraviolet radiation on the conidia of B. byssoidea*

Treatment (minutes)	Surviving spores		Percentage of variants among survivors
	Number[a]	Per cent	
0	130	100.00	0
1	122	93.85	0
2	116	89.23	0
4	60	46.15	0
8	42	32.31	0
16	18	13.85	0
32	2	1.54	0
64	2	1.54	0
128	0	0.00	0

[a] Represents the average number of surviving spores per plate from four replications.

which emitted 80 to 90 per cent of its energy at 2536Å was used. The plates were then incubated at 20° C. until the spores had germinated, and the germinated spores were transferred to test tubes. The cultures resulting from the treated spores were macroscopically identical to those of the untreated spores and produced a fluffy, white aerial mycelium.

There was a very low spore kill in the 1- and 2-min. treatments, but at 4 min. over half of the spores in each plate were killed, and the kill continued to increase until at the 32-min. treatment an average of 2 spores per plate survived.

Onions were inoculated with the cultures resulting from the treated spores and there was wide variability in the virulence of the different cultures. The amount of superficial mycelium produced on the diseased bulbs varied greatly. There also was variation in the number of spores produced on the bulbs by the different cultures. A few of the cultures sporulated very abundantly on the host and with scarcely any sign of mycelium. In most of the cultures a moderate amount of superficial mycelium developed, with little or no production of conidiophores on the host. One of the cultures formed flat, black sclerotia on the host, with neither conidia nor aerial mycelium during 12 days' incubation.

The mycelium from an 8-day-old culture was scraped into a Waring Blendor containing sterile distilled water and was cut into fragments 1 to 3 cells in length. One-half-ml. aliquots of this suspension were pipetted into Petri dishes on a thin layer of potato-dextrose agar. These plates were treated with ultraviolet radiation as described above. The hyphal

cells were killed readily by the ultraviolet ray treatment and after 8 min. of treatment every cell was killed. Hyphal-tip transfers were made from each of the surviving colonies, and each culture developed normal white, fluffy mycelium with no signs of sporulation.

The surface of 8-day-old cultures of *B. byssoidea* was scraped with a sterile needle until the mycelium was thoroughly macerated. These cultures were then exposed to ultraviolet radiation for 0.5, 1, 2, 4, and 8 min. They were incubated at 20° C. and examined at various intervals. The untreated cultures developed normally, while the treated cultures produced new white fluffy tufts of mycelium from the broken areas with no sporulation.

DISCUSSION

The usual wild type of *Botrytis allii* appeared to be very stable. There was no evidence of mutations of a mycelial type in repeated single-spore transfers from untreated cultures. It sporulated profusely and produced no sclerotia in culture. When mycelial tufts did occur they reverted to sporulation promptly after several mass transfers. Many mycelial variants were induced by exposure to nitrogen mustard, but after 4 to 5 mass transfers they reverted to the typical conidial type. Nothing was noted that gave any indication of a mutation of a permanent mycelial type suggestive of *B. byssoidea*. The atypical wild strain of *B. allii* was much more variable than any mutant induced by nitrogen mustard or observed to occur naturally in the typical strain. Various degrees of mycelium development and sclerotium formation in culture occurred. These variant cultures continued to vary in successive single-spore transfers with a tendency to stabilize after three or four spore generations. The cultures, however, did not resemble *B. byssoidea*, and the conidia always conformed closely to the measurements of typical wild *B. allii*. The variant *A5* of the atypical culture exposed to nitrogen mustard was the nearest of the variants to the typical wild type. It was distinctly more resistant to the chemical than typical wild *B. allii*, but at certain concentrations variants were induced. They were often mycelial, as were the variants in the typical strain, and tended to revert to the sporulating type promptly. Although the atypical wild type was much more variable naturally than the typical strain, it did not throw variants naturally or after exposure to nitrogen mustard that could be considered to resemble typical *B. byssoidea*.

Sporulation of *B. byssoidea* induced by growing on the host after each single-spore transfer did not yield any culture which sporulated on potato-dextrose agar. Sclerotia were produced readily in the typical strain of *B. byssoidea* on that media when incubated at 16° C. or below. A wide range of media did not induce sporulation except for very sparse, greatly delayed conidium formation in a synthetic medium in which sodium citrate or potassium oxalate was used as a source of carbon. Uranium nitrate in the medium produced only a temporary change in type of growth and in

sclerotium formation above 16° C. Variants were induced by nitrogen mustard when conidia from the host or when mycelial fragments from cultures were exposed. They were all mycelial, often showing excessive staling, and some were nonpathogenic. None showed any sign of sporulation. No variants were induced when spores or mycelium were exposed to ultraviolet irradiation.

This study of the two neck-rot pathogens indicates that they are very stable and have much less variability than many members of the Fungi Imperfecti. The conidial species and the mycelial species are apparently well fixed in their respective characters insofar as the typical forms are concerned. Mutations induced by such mutagenic agents as nitrogen mustard and ultraviolet radiation resulted either in degenerate forms or in rather rapidly growing mycelial thalli which gradually reverted to the original parental type.

It should be pointed out that in the case of several other fungi many of the variations induced by treatment with nitrogen mustard or by ultraviolet radiation have been stable through many transfers. Variant strains of *Penicillium* induced by mutagenic agents maintained their abnormal characters through repeated transfers (8). Abnormal cultural characteristics induced by treatment of *Phoma lingam* (Fr.) Desm. with nitrogen mustard were shown to persist through sub-cultures (1). The above species are uninucleate. When the multinucleate *B. allii* and *B. byssoidea* were exposed to nitrogen mustard many variants appeared but none maintained the new characters through repeated transfers. A possible explanation of the instability in induced variants of *Botrytis* may be found in the fact that it is multinucleate. If it is assumed that nitrogen mustard produces a change in the chromosomes of a single nucleus in a multinucleate spore, this spore could give rise to a culture with an altered morphological character immediately following the treatment. During subsequent growth the intermingling of nuclei through anastomosis might allow the formation of cells containing a full complement of nuclei like that of the original parent strain. Since the variant strains generally had a slower growth rate than the parent strain, it would be expected that hyphae arising from cells with a complement of normal nuclei would outgrow those from the variant strain containing mutant nuclei and would resemble the parent strain in cultural characteristics. On the basis of these observations on the reversion of induced variants of *Botrytis*, in contrast to the stability of similarly induced variants of uninucleate fungi, it might be expected that induced variants of other multinucleate fungi that readily undergo anastomosis would often revert to the original parent type.

SUMMARY

A study was made of natural and induced variability in two species of *Botrytis*. Typical wild *Botrytis allii* and *Botrytis byssoidea* produced

similar symptoms on the onion host except for greater sporulation of the former; but on culture media, *B. allii* sporulated abundantly, whereas *B. byssoidea* produced rank-growing, white, fluffy mycelium with seldom, if ever, any sign of sporulation. Both appeared to be very stable in culture. The conidia of *B. byssoidea* were slightly larger than those of *B. allii*.

Mycelial cultures of typical *B. allii* were obtained by making hyphal transfers from nonsporulating tufts and by treatment with nitrogen mustard (methyl-bis(β-chloroethyl)amine). However, these mycelial variants reverted to sporulation after several mass transfers, and no permanent cultures resembling *B. byssoidea* were produced.

A highly variable atypical wild strain of *B. allii* was isolated from onion only once. It readily produced mutants, and in the second single-spore generation a number of variant groups were formed which differed from one another by the presence or absence of sporulation, sclerotia, or appressoria. Cultures with characteristics similar to those of the respective variants were produced in the third single-spore generation.

Mutants of atypical *B. allii* induced by nitrogen mustard and those produced naturally showed a wide range of variability. However, none of these variants could be classified as *B. byssoidea*.

Nitrogen mustard and ultraviolet irradiation treatments of conidia and mycelium of *B. byssoidea* failed to induce sporulation, but produced variants with different growth rates and pathogenicity. Some variants showed a change in spore size, but they always remained within the range of *B. byssoidea*.

B. byssoidea was grown on a wide range of culture media, and sparse sporulation occurred only on a synthetic medium containing sodium citrate or potassium oxalate as a carbon source. Sclerotia developed at low temperatures (8° to 16° C.) on potato-dextrose agar, and at 24° C. when 0.25 to 0.75 per cent uranium nitrate was added to the agar.

Although atypical wild *B. allii* was extremely variable and a considerable range of variants was induced in typical *B. allii* and typical *B. byssoidea*, there were no indications that either species could be regarded as a variant of the other.

U. S. DEPARTMENT OF AGRICULTURE
AND
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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ANATOMICAL ASPECTS OF THE FUSARIUM WILT OF SWEET POTATOES[1]

T. T. McCLURE[2]

(Accepted for publication February 20, 1950)

The Fusarium wilt of sweet potatoes (*Ipomoea batatas* Poir.) is caused by *Fusarium oxysporum* f. *batatas* (Wr.) Snyd. et Hans., which invades the xylem and produces a brown vascular discoloration. Watanabe (6) reported that tyloses are formed in the xylem of diseased plants and that both tyloses and vascular discoloration may occur in advance of the invading mycelium.

Before the anatomy of diseased plants is discussed, that of healthy plants should be considered. Sweet potato sprouts of the Porto Rico Red variety were grown in a greenhouse and were examined under either a dissecting or a compound microscope. Paraffin sections were stained with safranin and fast green or by Cartwright's method (5).

THE ANATOMY OF HEALTHY PLANTS

When a sweet potato sprout is separated from its mother root and placed in moist steamed sand, the basal wound develops a callus by the proliferation of the thin-walled, parenchymatous cells adjacent to the wound. The proliferation is first evident in the region of the internal phloem and then in the regions of the pith and the external phloem. The xylem is passively enclosed by the overgrowth of the callus tissue developed from the internal phloem which fuses with that developed from the external phloem (Fig. 1, A). A meristematic layer of callus cells lies just beneath a surface layer of

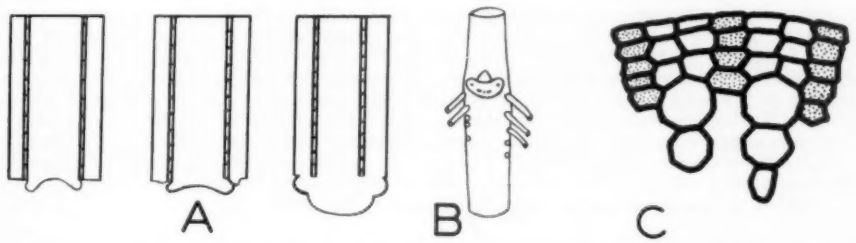

FIG. 1. A. Three stages in the development of a callus. Diagrams of longitudinal median sections of sweet potato stem bases, showing how the xylem (cross-hatched) is enclosed by overgrowth of callus. B. Diagram of a node of a sweet potato stem, showing relation of adventitious roots to leaf scar. C. Diagram of portion of transverse section of xylem of sweet potato stem, showing primary xylem (below), secondary xylem (above), and arrangement of xylem parenchyma cells (stippled).

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Present address: Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

thin-walled wound cork cells. A milky latex normally collects on the fresh basal wound of a sweet potato sprout and becomes amber-colored when dry. The presence of such dried latex does not suppress callus formation.

The sweet potato stem has a 2–5 phyllotaxy as reported by Hayward (3). Phyllotaxy of either clockwise or counterclockwise rotation were found, both types being common. Plants with either type were apparently equally susceptible to the disease.

The stem in the region of an internode has an unbroken siphonostele of secondary xylem with nine primary xylem strands internally adjacent to it. Just below a node, an arc of the stele bearing three primary xylem strands diverges to form the leaf trace. This arc breaks into three bundles, each containing one primary vascular strand, and then as a result of dichotomy

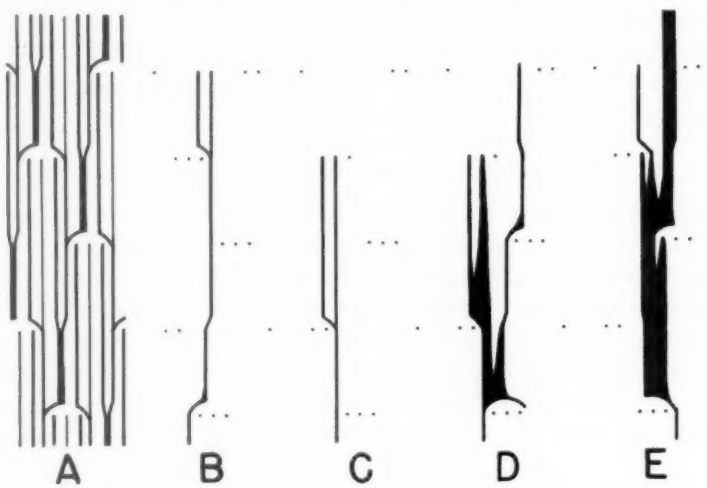

FIG. 2. A. Pattern of branching of the nine primary xylem strands in a sweet potato stem at five consecutive nodes. The two lateral edges of diagram join to form xylem cylinder. B–E. Patterns of vascular discoloration of stem obtained by inoculation of plants with the wilt Fusarium through a single severed root, the position of which in relation to the basal leaf is shown at bottom. The position of each leaf is indicated by three dots which correspond to the three primary xylem leaf traces, and is included to facilitate comparison with the pattern of the primary xylem (A). B and C. Pattern of vascular discoloration obtained after 2 weeks. D and E. Pattern of vascular discoloration obtained after 1 month. Note at basal nodes some circumferential spread of discoloration in addition to that provided by the primary xylem pattern.

of each lateral bundle, the five vascular bundles of the petiole are derived which were noted by Hayward (3). A piece of each end of the remaining arc of the stele diverges to form the vascular supply to the axillary bud, and the siphonostele is re-formed by the closure of the leaf-branch gap. Each of the two primary xylem strands which close the leaf-branch gap divides, making four strands, one of which undergoes dichotomy again at the next node. A diagram of the primary xylem pattern is shown in figure 2, A.

Adventitious root primordia originate in the external phloem, some of them prior to any stem injury, and occur in two rows which lie lateral to and extend basally from the attachment of the leaf to the stem (Fig. 1, B). The

first roots to appear at a node usually have five or six protoxylem strands. The primary xylem of the root connects with that secondary xylem of the stem which is lateral to the basal extensions of the leaf gap.

The secondary xylem siphonostele was found to be composed of thick-walled cells, all of which stain red with safranin and with phloroglucinol in hydrochloric acid. When the Maule test for lignin (5) was applied, certain radial rows of cells were not colored red. These cells have thick walls with simple pits, contain protoplasts, and are axially elongate. They adjoin similar cells at their ends as well as on their radial faces, thus forming radial tiers of cells. These tiers are one cell wide and frequently extend from pith to cambium and from node to node. Because of the axial elongation and continuity of these cells, they are considered in this paper to be xylem parenchyma rather than ray cells. The rest of the secondary xylem is composed of vessel elements which have a small simple perforation in each oblique end wall. The radial rows of vessel elements usually occur in tangential pairs which are separated from an adjacent group of vessel elements by a tier of xylem parenchyma (Fig. 1, C). In this way every water-conducting cell of the secondary xylem is radially adjacent to a tier of living cells and the path of water up the stem is divided into a number of channels which are more numerous than the primary xylem strands. With increase in girth of the stem, new xylem parenchyma tiers are formed which, because of their late origin, do not extend to the pith.

THE ANATOMY OF DISEASED PLANTS

One of the most obvious internal symptoms of plants infected with the wilt Fusarium is the brown discoloration of the vascular system. In severely infected plants the whole stele may be discolored, whereas in mildly infected plants only a part of the stele may be affected, giving the appearance of a brown streak in the otherwise white stele. The discoloration is less pronounced the greater the distance from the point of inoculation, and its distal limit is often impossible to determine. Isolations made from the stele at every inch up the stems of infected plants showed that the pathogen is present only in the most severely discolored areas, and that the discoloration precedes the pathogen up the stem.

Hyphae of the wilt Fusarium are typically found in vessels, and cells with protoplasts are invaded only after their death. Usually only one or two hyphae occur in each invaded vessel element. Plugging of the lumen with masses of mycelium was not observed. The walls of invaded vessel elements are golden-brown and frequently stain poorly with safranin. The discoloration is also found in the contiguous and proximate walls of adjacent parenchymatous cells and in the walls of those vessel elements which lie in advance of and in the same water channel as the invaded element.

Wound gum, which is also golden-brown but stains deeply with safranin, is often found as hemispherical masses which protrude into the lumen of an invaded vessel element (Fig. 3, A, g). These gum deposits are usually

located on half-bordered pits and are apparently secreted through the pits by the adjacent living cell. Bordered pits sometimes bear gum deposits, but in every case it was noted that a living tylosis was contiguous with the other face of the pit. Esau (1) found similar masses of wound gum in the xylem of alfalfa infected with the dwarf disease virus. Adjacent to the cambium, the youngest secondary xylem cells are often collapsed, and their lumens appear full of wound gum (Fig. 3, A, c).

Tyloses, which are seldom found in healthy plants, even as a response to sterile wounding, are abundantly formed in infected plants (Fig. 3, B). They are usually present in a vessel in advance of an invaded element and

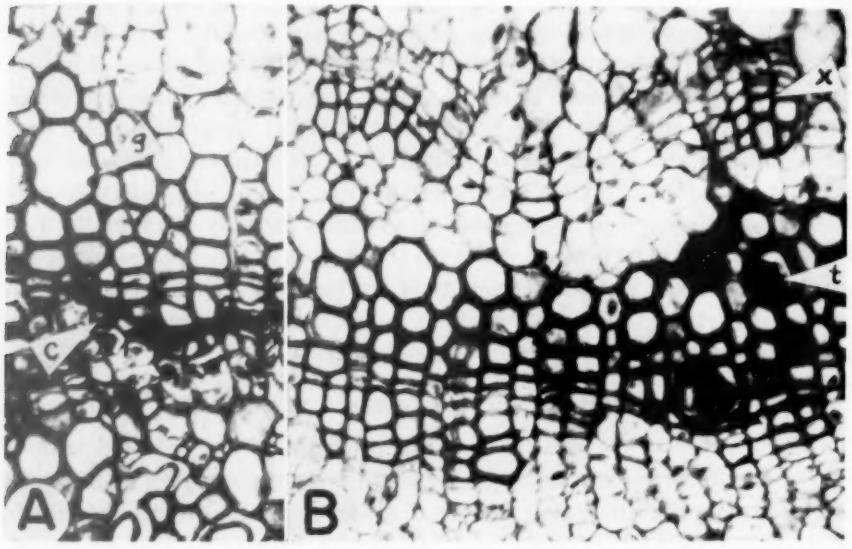

FIG. 3. Portions of transverse sections of wilt-infected sweet potato stems, oriented with pith at top. A. Hemispherical mass of gum on wall of vessel (g), and collapsed, gum-filled elements in youngest region of secondary xylem (c). B. Tyloses in vessels (t) and centripetal development of auxiliary xylem (x).

are sometimes also present in vessels adjacent to and in the same water channel as the invaded element. Tyloses have cellulose cell walls, are formed from adjacent living cells by extrusion through half-bordered pits, and are often so numerous and large that they become closely packed in the vessel lumen and lose their originally spherical shape. After staining by Cartwright's method, the walls of most of the tyloses are blue, except those nearest to the pathogen which are stained red. In this latter region a substance which appears to be wound gum accumulates in the interstices between tyloses and between tyloses and their enclosing vessel wall. The staining of the proximate tyloses with safranin may thus be due to wound gum secreted by them.

Penetration of a tylosis blockage or rupture of a tylosis by the wilt fungus has not been observed. Hyphae pass through vessel element apertures and through unobstructed pits but do not directly penetrate cell walls or

pits which are covered with wound gum. When occasionally a xylem parenchyma cell fails to produce wound gum and its protoplast dies, the pathogen may traverse the cell and invade the vessels of the next adjacent water channel.

An auxiliary secondary xylem is often found in infected plants and is developed from a cambium which arises in the region between the primary xylem and the internal phloem (Fig. 3, B). The first-formed auxiliary xylem elements lie adjacent to the primary xylem, and subsequent elements are developed in centripetal sequence, i.e., in the opposite direction of that found in the normal stele. Frequently associated with the formation of an auxiliary xylem is a longitudinal splitting of the stem, a symptom of the disease referred to by Harter and Weimer (2).

Infecting a sweet-potato sprout by dipping the fresh basal wound in a spore suspension of the pathogen results in a severe form of the disease in which no callus is developed, the basal wound is rotted, and the whole stele is discolored. Such plants usually die in approximately 2 weeks.

When healthy sprouts with fresh basal wounds are planted in infested soil, some may develop less severe symptoms than those just described. Some callus may be formed at one or more points on the basal wound, and the uncallused areas may rot. Failure of callus formation, or of fusion between internal and external calluses, is associated with infection of the stele at those points. The resultant vascular discoloration occurs as one or more streaks which are not uniform in either original width or position of origin in the stele.

Adventitious roots have been shown to arise at definite locations in relation to a node (Fig. 1, B), and their vascular connection with the stele has been described in the section on the anatomy of the healthy plant. As the pathogen requires a vascular wound for invasion, and as severed roots provide effective portals of entry (4), infection of the stele at a single predetermined locus is thus possible by the following method. Sprouts were set in sterilized sand until their basal and other wounds were healed. One adventitious root was then severed and the fresh wound exposed to a spore suspension of the pathogen. A mild infection resulted, involving only those water channels connected with the xylem of the severed root. These plants frequently showed the unilateral leaf symptoms described by Harter and Weimer (2), and survived for several months. When the streak of vascular discoloration was traced up the stem (Fig. 2, B-E), it was found to branch at the nodes in a manner very similar to the pattern of the primary xylem (Fig. 2, A). As the infection and discoloration was present in both the primary and secondary xylem, it is concluded that the water channels of the secondary xylem follow in a multiple manner the pattern of the primary xylem.

The circumferential spread of the pathogen at random in the xylem is apparently impeded by the tiers of living xylem parenchyma cells which radially separate the various water channels from each other. These cells are

generally successful in resisting invasion, although a few may die after prolonged proximity to the pathogen. Since these lateral barriers are but one cell wide, the eventual death of even one cell nullifies the delimiting effect of the whole tier by allowing the fungus to penetrate to a previously uninvaded water channel. The upward growth of the pathogen in the plant is not checked, despite the presence of effective tylosis blockages in many of the vessels. The fungus is able to by-pass tylosis blockages by growing through bordered pits into adjacent unclogged vessels. It is interesting that an unclogged vessel is apparently always accessible.

DISCUSSION

The presence of vascular discoloration in advance of the pathogen indicates that toxic by-products are produced and are carried in the transpiration stream. If it be postulated that these toxic products stimulate tylosis formation, and that in high concentration they damage living cells so that tyloses are not produced, then a hypothesis might be formulated to explain the unimpeded upward growth of the pathogen. In the vicinity of the fungus where the concentration of toxic products is presumably high, tylosis formation may be inhibited. As the toxic products are carried upward in the transpiration stream, the concentration decreases by diffusion to adjacent vessels, and tylosis formation is stimulated. As the number of tylosis-clogged vessels increases, the concentration of the toxic products in the remaining vessels increases until a concentration inhibitory to tylosis formation is attained, and thus an unclogged path may be provided by which the fungus can by-pass the clogged vessels.

The wound gum formed in half-bordered pits in the vicinity of the pathogen may act, physically or chemically, as a barrier which prevents hyphal penetration of the adjacent living cells. Wound gum may also play a part in preventing both the penetration of tyloses and the intrusion of the pathogen between the vessel wall and a tylosis wall.

SUMMARY

The basal wound of a sweet potato sprout normally develops a callus which covers the severed xylem by overgrowth. Callus formation is suppressed where the stele is infected with *Fusarium oxysporum* f. *batatas*.

An auxiliary xylem is often found in infected plants and is developed centripetally from an internal cambium. This development of new cells internal to the stele may be the cause of the stem splitting commonly found in Fusarium wilt-infected plants.

When the pathogen enters via a severed root, the pattern of vascular discoloration closely resembles the pattern of the primary xylem, even though both primary and secondary xylem may be invaded. Circumferential spread of the fungus in the xylem is restricted by the radial tiers of living xylem parenchyma cells which are normally present in the xylem and which resist invasion until they are eventually killed. Tyloses are formed

in advance of the fungus and are an effective barrier in the vessels in which they occur. The fungus grows around tylosis barriers by passing through bordered pits into unclogged xylem elements.

Division of Plant Pathology
University of California
Berkeley, California

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PHYTOPATHOLOGICAL NOTES

Verticillium Wilt in Acid Soils. STEPHEN WILHELM. The generalization made in a recent textbook of plant pathology[1] that Verticillium wilt does not occur on acid soils is open to considerable question. Van der Meer,[2] for instance, reported in 1925 that Verticillium attacks the potato in acid soils (pH 4.4 to 6.6) in Holland. It may be assumed from Van der Meer's paper that alkaline soils were not available in which to study Verticillium wilt. Haenseler[3] showed that yearly applications of sulfur to soil over a 4-year period caused a decrease in the severity of Verticillium wilt in eggplant, and that applications of lime caused an increase. In no instance did the sulfur applications give complete control, although they brought the soil reaction to a pH of between 4.2 and 4.8. For the most part the lime applications brought the soil reaction up only to the point of neutrality.

Martin[4] presented evidence similar to Haenseler's that liming increased the severity of Verticillium wilt in eggplant. The control obtained by sulfur applications was not, however, so striking. In sulfur-acidified plots having a pH of 4.6 to 4.8, 68.8 per cent of the plants became infected as compared with 77.1 per cent in the untreated plots and 98.6 per cent in the limed plots. The pH of the limed plots was 6.2. The pH of the untreated plots was not given, but presumably was between 4.6 and 6.2.

Though Verticillium wilt is a problem in alkaline soils in California, severe outbreaks occur repeatedly on soils well in the acid pH range. Verticillium infection was found in tomato in Solano County in soil of pH 6.4 and in San Joaquin County in soils of pH 4.6, 4.8, and 4.9; in strawberry in Santa Cruz County in soils of pH 6.0, 6.1, 6.5, 6.6, and 6.7; in cabbage in San Mateo County in soil of pH 6.6; in potato in Santa Cruz, Sonoma, and San Mateo counties in soil of pH 5.5, 5.3, and 5.9, respectively. Verticillium wilt was observed in a greenhouse tomato crop planted in soil of pH 4.9 used formerly for gardenias.

The pH determinations were made with a glass electrode on a saturated soil paste. Each pH mentioned above is the average of five separate determinations. To confirm the field identification of Verticillium wilt, isolations of *Verticillium albo-atrum* Rke. et Bert. were made from the vascular tissues of all plants under which soil was collected.

Verticillium wilt is a principal factor limiting the growing of strawberry, boysenberry, youngberry, and tomato in Santa Cruz County. Soils in this county range in general from pH 5.5 to 6.5. Nightshade (*Solanum nigrum* L.), which grows as a weed in irrigated orchard and truck crop land, also is commonly infected with Verticillium in this district.

[1] Chester, K. S. The nature and prevention of plant diseases. 584 pp. The Blakiston Co., Philadelphia. 1942.

[2] Van der Meer, J. H. H. Verticillium wilt of herbaceous and woody plants. Med. Landbouwhoogeschool te Wageningen 28: 1–82. 1925.

[3] Haenseler, C. M. Effect of soil reaction on Verticillium wilt of eggplant. New Jersey Agr. Exp. Sta. Ann. Rept. 41 (1927–1928): 267–273. 1928.

[4] Martin, W. H. Investigations of Verticillium wilt. New Jersey Agr. Exp. Sta. Ann. Rept. 44 (1930–1931): 50. 1931.

In the peat soils of San Joaquin County reactions as low as pH 4.5 are common. Some tomato fields in this district have 80 to 90 per cent of the plants infected with the wilt Verticillium.

Cabbage and Brussels sprouts,[5] grown extensively in San Mateo and adjoining coastal counties in soils usually well in the acid pH range, have been severely affected by Verticillium wilt.

It is concluded that the occurrence and severity of Verticillium wilt is not greatly affected by soil reaction within the range in which susceptible crops are commonly grown. Severe outbreaks of Verticillium wilt in a variety of crops occur repeatedly on soils well in the acid pH range. Though not all soil series of California have been investigated in this connection, no series important for growing of crops has been found in which Verticillium wilt may not occur.—Division of Plant Pathology, University of California, Berkeley, California.

The Value of Copper Fungicides for the Control of Strawberry Leaf Spot.[1] E. G. SHARVELLE AND M. N. TELLER.[2] Leaf spot of strawberries, caused by *Mycosphaerella fragariae* (Tul.) L., appears annually in most strawberry plantings in Minnesota. The exact losses from this disease have not been accurately determined for this area but it is reasonable to assume that the vigor of affected plants and their ability to withstand the rigorous winters of Minnesota might be materially affected by a high incidence of leaf-spot infection. It is further possible that continual recurrence of leaf-spot infections in perennial plantings of strawberries may reduce the yield of berries significantly. It is doubtful that strawberry leaf spot materially reduces the current season's crop in Minnesota, because the peak of leaf-spot infection usually is attained after the crop has been harvested.

During the summer of 1944, the value of fungicidal sprays containing copper for the control of strawberry leaf spot was determined in plots of the variety Senator Dunlap at University Farm, St. Paul, Minnesota. Size of a spray plot varied slightly because of variable stands throughout the planting, but areas of comparable uniformity were selected and three replicates were used for each spray treatment. Two sprays, applied with a knapsack sprayer, were used on each plot. The first was applied June 9, when flower petals were beginning to drop; the second was applied June 21, when all petals had fallen and most of the fruit had set. Incidence of leaf spot on individual leaflets and on entire leaves was recorded August 19. Fungicidal injury to strawberry foliage was scored on a scale of 0 to 3 according to the percentage of leaves injured.

[5] Snyder, William C., H. N. Hansen, and Stephen Wilhelm. New hosts of *Verticillium albo-atrum*. U.S. Dept. Agr., Plant Dis. Rptr. 34: 26–27. 1950.

[1] Published with the approval of the Director as paper No. 655 in the Miscellaneous Journal Series of the Minnesota Agricultural Experiment Station.

[2] The present address of the senior author is Purdue Agricultural Experiment Station, Lafayette, Indiana; that of the junior author is Parke, Davis and Company, Detroit, Michigan.

The leaf-spot fungus infected 65.2 per cent of the leaves of the control plants, with each leaf bearing an average of more than four spots. Yellow Cuprocide was most effective in reducing leaf-spot infection but it was most injurious to the strawberry foliage (Table 1). Only 24.4 per cent of the

TABLE 1.—*The control of strawberry leaf spot by fungicidal sprays containing copper and the foliage injury caused by the fungicides*

Fungicide	Percentage of leaves infected[a]	Average number of leaf spots on a leaf	Percentage of leaves injured by spray	Injury index[b]
Control	65.2	4.3	0.0	0
Yellow Cuprocide				
3 lb. in 100 gal.	24.4	2.5	58.4	3
6 lb. in 100 gal.	29.2	2.5	65.7	3
Microgel				
3 lb. in 100 gal.	40.1	3.3	40.0	1
6 lb. in 100 gal.	46.3	3.8	49.7	2
Bordeaux				
4–4–50	33.7	2.8	54.8	2+
Tenn. Copper 34				
3 lb. in 100 gal.	45.3	4.0	44.3	1
6 lb. in 100 gal.	33.5	3.0	47.7	2

[a] Each figure is the average for duplicate or for triplicate plots.
[b] The higher numerical values indicate greater injury.

leaves were infected when this fungicide was used at 3 lb. in 100 gal. Increasing the rate to 6 lb. in 100 gal. did not improve control of leaf spot and resulted in greater injury to the foliage. Tennessee Copper 34 at 3 lb. in 100 gal. was not sufficient to greatly reduce leaf-spot infection, and even at 6 lb. in 100 gal. it was less effective than Yellow Cuprocide. Bordeaux 4–4–50 was no better than the Tennessee Copper 34 at its higher rate and was somewhat more injurious to strawberry foliage. Microgel was inferior to the other materials in controlling disease, but it and the Tennessee Copper 34 were less injurious to foliage than were Yellow Cuprocide or Bordeaux. None of these materials can be recommended as a substitute for tribasic copper sulphate.—Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

Control of Brown Rot.[1] E. G. SHARVELLE AND M. N. TELLER.[2] Introduction of cherry-plum hybrids has formed the basis for a limited stone fruit industry in Minnesota. Relatively few acres of this hybrid were grown in 1945, but larger acreages of stone fruits may result from the introduction of Korean (*Prunus japonica* Thunb.) and Nanking cherries (*Prunus tomentosa* Thunb.) and sour cherries. Brown rot, caused by species of *Sclerotinia,* is the principal disease of stone fruits in the State. Blossom and twig blight and fruit rot occur frequently.

In the summer of 1945, trees of the Sapa variety of the cherry-plum

[1] Published with the approval of the Director, Minnesota Agricultural Experiment Station, as Paper No. 2284 in the Scientific Series.

[2] The present address of the senior author is Purdue Agricultural Experiment Station, Lafayette, Indiana; that of the junior author is Parke, Davis and Company, Detroit, Michigan.

hybrid in a commercial orchard[3] were sprayed with various fungicides to control brown rot. Single cover sprays of the following materials were applied 10 days prior to harvest: Dritomic Wettable Sulphur, 4 lb. in 100 gal.; Dritomic Wettable Sulphur, 3 lb. in 100 gal., plus Tennessee Copper 34, at 1 lb. in 100 gal.; Fermate (ferric dimethyl dithiocarbamate), 1 lb. in 100 gal.; and a commercial lot of Fermate used at the same rate. Isolated plantings of the hybrid in the vicinity of the commercial orchard were considered as controls.

Fruit infection in the orchard was determined just prior to harvest. Duplicate samples of 1000 fruits from a spray plot were examined for brown rot. Infection on the near-by control trees was 80 per cent. In the commercial orchard, only 4.1 per cent of the fruits from the Wettable Sulphur plot were infected, and only 1.1 per cent of those from the plot sprayed with Wettable Sulphur plus Tennessee Copper 34. The two lots of Fermate resulted in only 1.7 and 1.8 per cent of the fruits being infected. Thus, all of the fungicides used satisfactorily controlled fruit infection in the commercial orchard when there was sufficient inoculum in the vicinity to cause 80 per cent infection in trees that were not sprayed.

Lots of the apparently healthy fruits from each spray plot were spread on trays and placed in storage. The experience of Minnesota growers has been that although the fruits apparently are free from brown rot at harvest time they invariably break down rapidly during bulk storage, when brown rot can spread rapidly from fruit to fruit. The samples from the sprayed plots were examined daily during the first 2 weeks of storage to determine how much protection the fungicides afforded for the fruits during storage.

The sample from the Wettable Sulphur plot deteriorated rapidly. At the end of the first week in storage 25.7 per cent of the fruit was rotting, and within 2 weeks 61.4 per cent was destroyed by brown rot. Additions of Tennessee Copper 34 to the Wettable Sulphur spray had reduced the loss from brown rot in storage. Only 21 per cent of the fruits in this sample was destroyed by brown rot during 2 weeks in storage.

Although both lots of Fermate had controlled brown rot infection of fruits in the orchard, the commercial lot of Fermate was less effective than the experimental lot of the chemical in preventing fruit rot in storage. During 2 weeks in storage, 39.6 per cent of the fruits from trees sprayed with the commercial compound had decayed while only 16.7 per cent of the fruits from trees sprayed with the experimental lot were destroyed by brown rot.

Other chemicals were tested for their fungicidal value on picked fruits stored at 25° C. Two hundred apparently healthy fruits were placed on brown paper in each of six wooden greenhouse flats. Lots 1 and 2 were dusted with experimental copper dusts A and B, respectively; lot 3, with Apple Dritomic Sulphur; lot 4, with Chloramine B; lot 5, with Fermate;

[3] The authors express their appreciation to the Andrews Nursery, Faribault, Minnesota, for the use of their commercial orchard.

and lot 6 was used as a nontreated control. In each case, enough dust was applied with a small puff-duster to insure a fine coating on each fruit. A cheesecloth cover for each flat kept away insects.

The fruits were inspected 48 hr. after the dusting and then daily for the next 10 days. Infected fruits were immediately discarded. At the first inspection none of the fruits dusted with Chloramine B was infected with brown rot, but from 3 to 9 per cent of fruits dusted with other chemicals were infected with the *Sclerotinia*. In the control lot, 24 per cent of the fruits had brown rot. At the final inspection 50 per cent of the fruits treated with Chloramine B had been infected and discarded. The other dusts were even less satisfactory because 77 to 78.5 per cent of the fruits had been infected and discarded. At the last inspection of the control lot, 72 per cent of the fruits had been destroyed by brown rot and discarded.

These fruit samples, which appeared healthy when selected and stored, must have been infected in the field prior to the chemical dust treatments. The experiment, therefore, must be considered as a test for the eradicant value of the chemicals rather than as a test for protective values.

The Chloramine B dust, which was promising in the first few days of storage at 25° C., soon lost its effectiveness. Although it slightly reduced loss from brown rot, the results were not such as to warrant its recommendation.

At the present time, brown rot on fruits of the Sapa variety of cherry-plum hybrid can be controlled in the orchard, but there is no satisfactory means of preventing storage losses from brown rot.—Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

Flower Blight of Stephanotis floribunda, Caused by Botrytis elliptica, and Its Control. C. M. TOMPKINS AND H. N. HANSEN. A severe infection of flower buds and open flowers of *Stephanotis floribunda* Brongn., grown in raised greenhouse beds, was first observed in a nursery at San Bruno, California, in July, 1945. More than 2,000 clusters of the attractive white, waxlike flowers, used principally for corsages and bridal bouquets, were rendered unsalable.

The disease is first observed on the white buds and open flowers in the form of numerous, irregular-shaped, pink to claret-colored spots, ranging in size from mere flecks to small lesions 1 to 5 mm. in diameter. Later, some lesions may coalesce, frequently involving, in the aggregate, one-half or more of the entire surface of the bud or open flower. The disease causes marked distortion.

Naturally infected flower buds and open flowers of *Stephanotis floribunda* were thoroughly washed in tap water and surface-disinfected for 2 min. in a 1–1,000 solution of mercuric chloride, rinsed three times in sterile distilled water, and dried on filter paper. Tissue fragments were then planted on poured plates of acidified potato-dextrose agar. All plates consistently yielded a fungus which was identified as *Botrytis elliptica* (Berk.) Cooke.

Four small, healthy, potted Stephanotis plants in flower were placed in a cool greenhouse (13° to 19° C.), watered thoroughly, and provided with clay saucers filled with water. There were five clusters of flower buds and open flowers on each plant. The clusters on three plants were inoculated by atomizing them with a spore suspension of the fungus. The fourth plant was atomized with sterile distilled water and served as the control. Immediately after atomizing, the plants were covered with glass bell jars to provide the necessary humidity.

After 4 days, 34 of 54 buds and open flowers on the three inoculated plants became infected, whereas the 19 buds and open flowers on the control plant remained healthy. The fungus was reisolated in pure culture from representative samples taken from buds and open flowers on each of the three infected plants and proved to be identical with the original isolate. The reisolates were pathogenic. The symptoms produced on inoculated flowers were identical with those occurring under natural conditions.

Artificial infection was obtained in the greenhouse in 3 days on red and on white flowers of florist's cyclamen (*Cyclamen indicum* L.) which had been atomized with a spore suspension of the Stephanotis isolate. The lesions were roughly elliptical, 1 to several millimeters in diameter, necrotic, and caused distortion of the petals. The flowers of *Gardenia jasminoides* Ellis, *Bouvardia humboldtii* Hort., tuberous-rooted begonia (*Begonia tuberhybrida* Voss), *gloxinia* (*Sinningia speciosa* Benth. and Hook.), yellow calla (*Zantedeschia elliottiana* Engler), *Caladium bicolor* Vent., *Dieffenbachia picta* Schott, *Lilium elegans* Thunb., *Philodendron cordatum*, and leaves of Los Angeles head lettuce (*Lactuca sativa* L. var. *capitata* L.) failed to become infected after inoculation.

MacLean[1] reported two nonliliaceous hosts for *Botrytis elliptica*, namely, autumn crocus (*Colchicum autumnale* L.) and seedling stock of *Gladiolus* sp. Later, MacLean and Shaw[2] found the fungus associated with a stem, leaf, and petal spotting of *Erythronium grandiflorum* in Washington.

Botrytis blight of Stephanotis flowers has not reappeared in the greenhouses at San Bruno since its initial appearance in the summer of 1945. Toward the end of the flowering season that year, the ventilators were closed and higher air temperatures restored. This procedure definitely inhibited the development of the disease on the last crop of flowers and proved to be an efficient means of control. It is now a standard cultural practice to keep the greenhouse ventilators closed during the Stephanotis flowering season. Attempts to discover the source of inoculum were unsuccessful.—Division of Plant Pathology, University of California, Berkeley, California.

[1] MacLean, Neil Allan. New hosts for *Botrytis elliptica*. Phytopath. 38: 752–753. 1948.

[2] MacLean, Neil Allan, and Charles Gardner Shaw. New hosts for *Botrytis cinerea* and *B. elliptica* in the Pacific Northwest. Phytopath. 39: 949–950. 1949.

Effect of Starving Infective Aphids on the Transmission of the Beet Yellow-net Virus. EDWARD S. SYLVESTER. Watson[1] demonstrated that the transmission of a persistent (beet yellows) and a nonpersistent (beet-mosaic) virus was affected if infective aphids were starved following an infection feeding, prior to the test feeding. In both cases the vector efficiency was reduced, and the reduction was notable after the first hour of starvation. The initial loss of infectivity was greatest in the case of the beet-mosaic virus (nonpersistent), and in the case of the beet-yellows virus (persistent) the greatest loss was during the first 5 hr. of starvation.

The vector-virus relationships of the beet yellow-net virus (persistent) and the green peach aphid are similar to the relationships of the beet-yellows virus and the green peach aphid.[2] However, the effect of starvation after the infection feeding has not been reported for the beet yellow-net virus–green peach aphid relationship.

To test this point, infective green peach aphid apterae, *Myzus persicae* (Sulzer), reared on a diseased yellow-net plant, were starved in a vial for periods of 4, 8, 12, and 24 hr. Single individuals were then tested upon healthy beet seedlings for infectivity. An equal number of unstarved infective individuals were tested as a check. The results of two trials are in table 1.

TABLE 1.—*Ability of single infective green peach aphid apterae to retain the beet yellow-net virus during various starvation periods after the infection feeding*

	Infectivity[a] after starvation period of				
Trial	0 hr.	4 hr.	8 hr.	12 hr.	24 hr.
I	6/30	10/30	9/30	.....	.....
II	18/30	.....	.....	21/30	14/30

[a] The numerator is the number of plants infected after a 24-hr. test feeding; the denominator, the number of plants inoculated.

The results obtained indicated that starving infective aphids, for periods under 24 hr. in duration, had little or no effect on the ability of the green peach apterae to retain or transmit the beet yellow-net virus. Apparently the vector-virus relationships for the aphid-borne persistent viruses may vary considerably.—Division of Entomology and Parasitology, University of California, Berkeley, California.

Blackpatch of Soybean and Other Forage Legumes.[1] J. L. WEIMER.

[1] Watson, M. A. The transmission of beet mosaic and beet yellows viruses by aphides; a comparative study of a non-persistent and a persistent virus having host plants and vectors in common. Proc. Roy. Soc. Lond., B, 133: 200–219. 1946.

[2] Sylvester, E. S. Transmission of sugar-beet yellow-net virus by the green peach aphid. Phytopath. 39: 117–132. 1949.

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Georgia Experiment Station, Experiment, Georgia. Paper No. 191, Journal Series, Georgia Experiment Station.

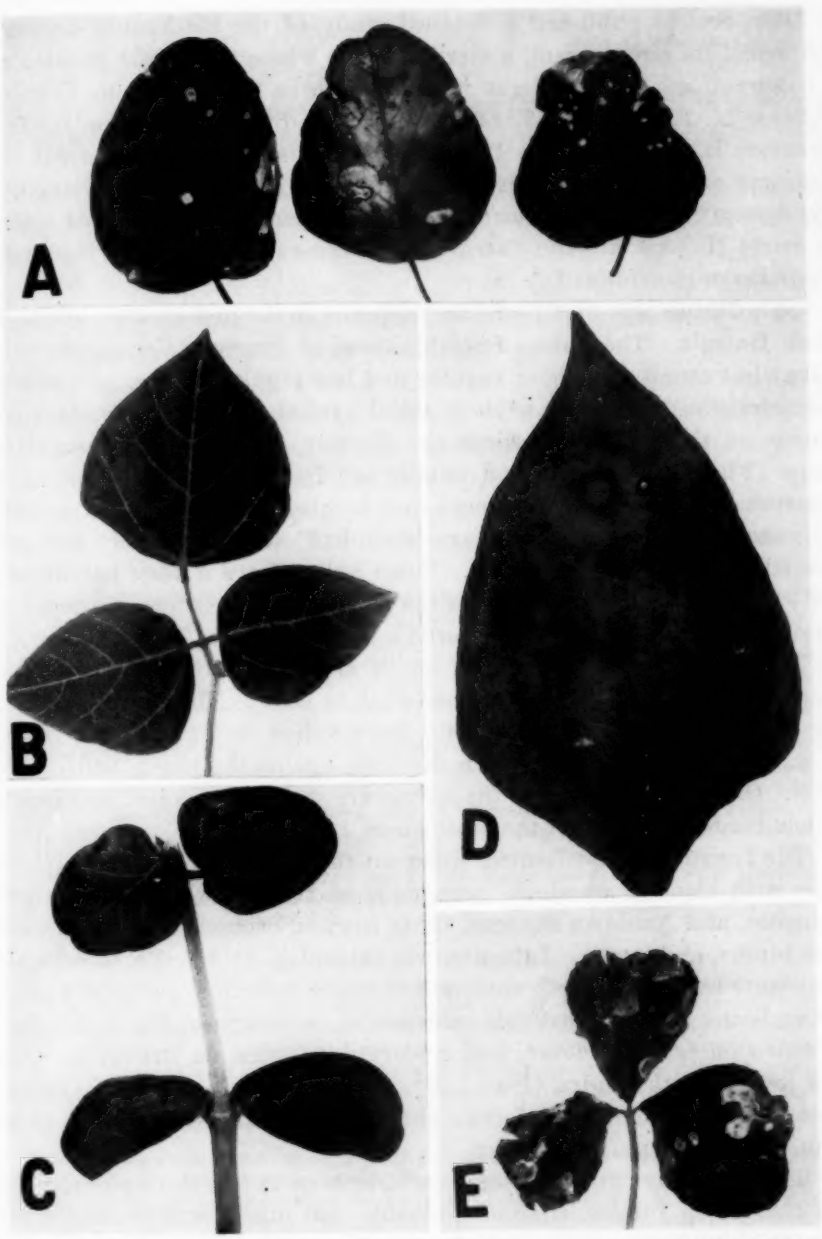

FIG. 1. Lesions on different hosts resulting from inoculation with the blackpatch fungus isolated from soybean. All plants were inoculated February 24, 1948, but not all were photographed on the same date. The hosts and dates photographed are: A. Ogden soybean photographed March 29, 1948. $\times \frac{3}{8}$. B. Kudzu photographed February 27, 1948. $\times \frac{7}{10}$. C. Palmetto soybean photographed February 27, 1948. $\times \frac{3}{4}$. D. Groit cowpea photographed March 2, 1948. $\times \frac{7}{10}$. E. Crimson clover photographed March 2, 1948. $\times 1\frac{3}{5}$.

In 1937, Smith[2] published a detailed study of the blackpatch disease of clover and its causal agent, a sterile fungus whose taxonomic position was not determined. This fungus was found to be pathogenic on *Trifolium pratense* L., *T. repens* L., *T. repens* L. var. Ladino, *T. hybridum* L., *T. incarnatum* L., *Melilotus alba* Desr., *M. officinalis* Lam., *M. indica* All., and *Medicago sativa* L. The purpose of this note is to give a brief report of this disease on soybean (*Glycine max* (L.) Piper), cowpea (*Vigna sinensis* (Torner) Hassk.), kudzu (*Pueraria thunbergiana* Benth.), and blue lupine (*Lupinus angustifolius* L.).

This disease was first found on soybeans in August of 1948 at Experiment, Georgia. The lesions resemble those of frogeye (*Cercospora sojina* Hara) but usually are more angular and less regular in size, and have the characteristic dark-brown-to-black aerial hyphal strands of the blackpatch fungus on the surface. Lesions are circular, ellipsoidal, or irregular in shape (Fig. 1, A and C), and usually are from 1 to 5 mm. in diameter. Sometimes adjacent lesions merge and involve half or more of a leaflet. On young soybeans, the lesions are cream-buff[3] at the periphery and grade towards buff-olive at the center. Some lesions have a very narrow sepia border on the upper surface. On the cotyledons the lesions are more uniform in color, varying from Saccardo's umber to sepia (Fig. 1, C).

The lesions on the other hosts are of approximately the same size and shape as on soybean but differ somewhat in color. The upper surface of the lesions varies from nearly white, ivory yellow, or light buff to Dresden or mummy brown, depending on the host, age of the plant, and environment. On kudzu (Fig. 1, B) the lesions are sharply delimited and lack the yellow border common on the other hosts.

The fungus has not fruited either on the host or in culture. Inoculations with blended mycelium were made on the following plants: Ogden, Palmetto, and Arcadian soybean, Groit cowpea, crimson clover, red clover, blue lupine, and kudzu. Infection was evident in 48 hr. Three days after the plants were inoculated, moderate to heavy infection was evident on all the soybeans, slight to moderate infection on the cowpeas (Fig. 1, D), heavy infection on crimson clover, and moderate infection on the lupine. Only new leaflets of the kudzu (Fig. 1, B) plants were infected. Infection was most abundant on the cotyledons and younger leaflets, especially in soybean, lupine, cowpea, and kudzu.

Since so many species of legumes have been shown to be susceptible to the blackpatch fungus, it seems probable that others will be found to be susceptible under conditions suitable for the rapid growth of the fungus.—Georgia Experiment Station, Experiment, Georgia.

[2] Smith, O. F. A leaf spot disease of red and white clovers. Jour. Agr. Res. [U.S.] 54: 591–599. 1937.

[3] Ridgway, R. Color Standards and Nomenclature. 43 pp. Illus. Washington, D. C. 1912.

REPORT OF THE 1950 ANNUAL MEETING OF THE SOUTHERN DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1950 annual meeting of the Southern Division of The American Phytopathological Society was held as a section at the meeting of the Association of Southern Agricultural Workers, February 9, 10, and 11, in Biloxi, Mississippi. A joint session was held with the Crops Division of the Agronomy Section. More than 75 plant pathologists were in attendance, and 33 formal papers were presented.

A business session was held on the morning of February 11, when the following officers were elected: *President,* W. A. JENKINS; *Vice-President,* PHARES DECKER. The offices of *Secretary-Treasurer* and *Councilor* are administered by J. A. LYLE and A. L. SMITH, respectively.

Titles and abstracts of papers presented follow.

JAMES A. LYLE, *Secretary-Treasurer*

The Fusarium Wilt of Crotalaria. ARMSTRONG, JOANNE K., AND G. M. ARMSTRONG. Single-spore cultures of a crotalaria-wilt *Fusarium* were obtained from Louisiana, Mississippi, Florida, and India, and used to inoculate plants of seven species of crotalaria in greenhouse experiments. Biological races were found on the basis of selective pathogenicity, numbers 1, 2, and 3 from India, the Louisiana-Mississippi area, and Florida, respectively. Wilt Fusaria from cotton, okra, tomato, and cowpeas produced no external symptoms of wilt in plants of one or more species of *Crotalaria.* One wilt-Fusarium isolate from crotalaria produced appreciable internal discoloration in cowpeas, and other crotalaria isolates produced even greater discoloration in *Cassia tora* L. The Louisiana-Mississippi isolates caused only 11.5 per cent external wilt of the Yelredo soybean and the Florida isolate caused only 14.3 per cent wilt of the L Z soybean. No wilting occurred in sesame, sweet potato, tobacco, and pigeon-pea plants inoculated with one or more crotalaria-wilt isolates. There were appreciable differences in the morphological and cultural characters of the Indian and American Fusarium isolates, but they fall within the range of *Fusarium udum* var. *crotalariae* Padwick and this name is accepted for the American fungus.

A Fusarium Wilt of Sesame in the United States. ARMSTRONG, JOANNE K., AND G. M. ARMSTRONG. A Fusarium wilt of sesame (*Sesamum indicum*) was found in south Georgia in 1948. Several of the monoconidial isolates of *Fusarium* and several varieties of sesame have been used in experiments in the greenhouse. One variety of sesame was wilt-resistant, others were apparently intermediate in resistance, and several were very susceptible. Inoculations of sesame with wilt Fusaria from 19 genera and species of plants and the inoculation of most of these plants with the sesame-wilt *Fusarium* gave negative results.

Helminthosporium victoriae as a Leaf-Spotting Pathogen. ATKINS, J. G., JR. In addition to the invasion of young seedlings and basal nodes of susceptible oat varieties in Louisiana, the Helminthosporium or Victoria blight pathogen, *H. victoriae,* also attacks and produces leaf spots on the leaves of plants approaching maturity. The leaf spots result from local infection and are distinct from the foliar striping of seedlings. Spots are oval to elongate, gray to brown, and up to 1×3 cm. in size. Conidia from leaf spots are distinguished from the typical conidia produced on basal nodes by their lighter color, more uniform length and septation, and slight curvature. Leaf spots were found on susceptible varieties before nodes were blackened and lodging occurred. Nearly all of the leaves of plants in the heading or dough stage may be killed by the pathogen, alone or in combination with rust. *H. victoriae* was consistently associated with the leaf spots. The fungus was isolated from leaf, sheath, and nodal tissues. All isolates were culturally similar and pathogenic on seedlings of Victorgrain oats. Inoculation of large oat plants resulted in leaf spots similar to those observed in the field.

Studies of Southern Bean Mosaics in Mississippi. BAIN, DOUGLAS C. A pod-mottling and distorting virus disease of beans is very common in poorly drained areas of fall-planted Stringless Black Valentine in the Crystal Springs area. Mottling on some or all pods of a plant may be similar to that of Bean Mosaic Viruses 4 and 4A. Varietal reaction and time for symptom expression at low temperatures differ. Local infection occurred on the varieties Stringless Red Valentine, Top Notch Golden Wax, Giant Stringless Green Pod, Bountiful, Stringless Refugee, Contender, Logan, Idaho Refugee, U. S. No. 5 Refugee, White Half Runner, and striped Creaseback; systemic infection on French Horticultural and Pencil Pod Wax; no infection on Woods Prolific Lima, Henderson Bush Lima, or Florida Belle. Systemic symptoms, without pod mottling, occurred on Full Measure, Dwarf and London Horticultural, and Plentiful. No infection occurred on other species. Local and systemic symptoms appear in 5 and 10 days, respectively, at 16° C. Local symptoms appeared on Ideal Market when inoculated with juice heated to 93° C. for 10 min. or diluted 1–500,000. Systemic symptoms (pod mottling) were obtained in Stringless Black Valentine with juice heated to 80° C. or diluted 1: 1000. Limited tests indicate the virus is not seed-borne or soil-borne. It is possibly a strain of Bean Mosaic Virus 4 or 4A.

Cotton Leaves as a Factor in Disseminating Verticillium albo-atrum. CHILTON, J. E., AND L. A. BRINKERHOFF.

Bordeaux Mixture Alone or Bordeaux Mixture Followed by Ziram May be Used to Control Pecan Scab. COLE, JOHN R. During the 1949 season, control of scab (*Cladosporium effusum*) on the Schley variety of pecan was extremely difficult, especially in the Fort Valley, Georgia, area, with the regular spray schedule—i.e., four applications of Bordeaux mixture or of ziram. Above-normal rainfall during March through August caused unusually severe incidence of the disease at Fort Valley. Because of a light set of nuts on the Moore variety in plots at Monticello, Florida, only two applications of Bordeaux mixture or of ziram were needed for control on pecan foliage. At Albany, Georgia, six applications (three of Bordeaux followed by three of ziram) gave commercial control on Schley nuts and foliage. At Fort Valley, Georgia, four applications of low-lime Bordeaux or one application of Bordeaux and four of ziram gave poor control on both foliage and nuts of Schley. Likewise, because of early infection with scab, the pink-rot fungus (*Cephalothecium roseum*) gained entrance through scab lesions and caused considerable damage to kernels. Most commercial varieties extensively planted in orchards in the southeast are susceptible to pecan scab. Listed in order of susceptibility they are: Schley, Success, Pabst, Frotscher, Teche, Mobile, Moneymaker, Moore, Brooks, Farley, Desirable, and Stuart. Concentrated sprays have been used to a limited extent but are still in the experimental stage. Pecan growers should continue to use the usual hydraulic spray machinery to apply Bordeaux mixture or ziram during the season of 1950 in order to control pecan diseases.

Root Knot of Peanuts. COOPER, W. E. An examination of peanut plants in June, 1949, from an area in which the plants were severely stunted and dying, revealed that the roots were discolored, short, and matted and had numerous small galls. These galls were from 1 to 3 times the diameter of normal roots and contained from one to four female root-knot nematodes. Later, nematodes were found in roots of severely affected plants from five North Carolina counties. Occasionally nematode galls were found on plants with normal top growth. After all plants were removed from an area with severely stunted plants, the rows were treated with Dowfume W-40 and Dowfume N at 2.7 and 4.7 ml. per linear foot. Peanut seeds were planted in these rows 14 days later. No differences in stand resulted; however, after 10 weeks' growth the plants from the treated rows were practically free of galls and weighed from 50 to 95 per cent more than the controls. The relationship of this nematode to others reported on peanuts is not known, but apparently it is not the strain which commonly attacks tobacco. Infestation by this peanut root-knot nematode may be a factor causing poor plant growth, especially when peanuts follow peanuts.

Some Notes on Diseases of Blue Lupine. DECKER, PHARES. Among the diseases of blue lupine, *Lupinus angustifolius* L., normally occurring in Florida, *Rhizoctonia* spp. and *Sclerotium rolfsii* Sacc. may cause serious seedling losses, especially when warm dry periods follow seeding. *S. rolfsii* may continue to kill plants in an extended warm period during the growing season. Anthracnose, *Glomerella cingulata* (St.) S. & S., may cause stem cankers in a warm fall, but is most active in reducing the seed crop when prolonged warm, humid periods occur at flowering and until seed maturity. Losses from brown spot, *Ceratophorum setosum* Kirchn., have been observed only during cool, wet winters. Mildew, *Erysiphe* sp., may cause defoliation and a reduced seed crop following unusually mild winter weather. *Botrytis cinerea* and *Sclerotinia sclerotiorum*

(Lib.) Masse. may be found each season but have caused only moderate losses. The propagation of individual plants selected from commercial field plantings indicates that many plant types, seed-coat markings, and disease-reacting strains come true from seed. Strains of lupine more susceptible than the commercial strains to *Rhizoctonia* and *S. rolfsii* have been isolated. Strains have been established that produce, in spite of brown spot and anthracnose, more green weight and a heavier seed crop than does the certified commercial seed. The variation in disease reaction appears to be klendusic in nature rather than due to an inherited plant characteristic.

Chemical Seed Treatments for Blue Lupine. DECKER, PHARES. In greenhouse experiments, chemical seed treatments decreased seedling losses of blue lupine, *Lupinus angustifolius* L., when plantings were made in soils infested with *Rhizoctonia* and *Sclerotium rolfsii* Sacc. When plantings were made in naturally infested soil in the field, a small but significant (5 per cent level) increase in stands usually resulted from chemical treatment. Environmental conditions prevailing from seeding to the hardening of the seedling plants determine stands to a much greater degree than do chemical seed treatments. No chemical seed treatment has been found that will insure a good stand of blue lupine under environmental conditions adverse to the host. Chemicals were applied to a lot of seed containing 20 per cent of anthracnose-infected seed. Replicated plantings were made at Auburn, Alabama, Tifton and Experiment, Georgia, and at Gainesville, Quincy, and Milton, Florida. Judged by plant emergence counts and stand counts 2 weeks after emergence, no single chemical was best at all locations. Stand of the untreated seed was intermediate at several locations. Phygon, Spergon, Arasan, Ceresan 2 per cent, Ceresan M, Red Copper Oxide, Dow 9B, and Dow F-800, used as chemical seed-treating materials at the rate of 1–2 oz. per 100 lb. of seed may reduce, but will not prevent, the nodulation of the lupine plants.

The Use of Fungicides on Downy-Mildew-Resistant Cucumber Varieties. EPPS, WILLIAM M. Downy mildew on fall cucumbers in coastal South Carolina is normally so severe that its control on susceptible varieties by the application of fungicides has been difficult and frequently impossible. After the introduction of the mildew-resistant Palmetto variety the question was raised concerning its degree of resistance and its need of fungicides for mildew control. In tests conducted between 1946 and 1949, mildew was sufficiently severe on Palmetto so that the application of fungicides resulted in increased yields only where Palmetto was interplanted with a susceptible variety. Dithane Z-78 (zineb) gave the best control under these conditions, followed by Zerlate (ziram) and Tribasic Copper Sulfate. Parzate (zineb and nabam) was ineffective. Where Palmetto was isolated from susceptible varieties or even planted in a block adjacent to susceptible varieties, mildew was not a problem even in the undusted plots. Palmetto is resistant to but not immune from mildew. A few lesions always appeared but they produced few if any sporangia. Thus secondary spread occurred in Palmetto only to a limited extent or not at all, and damage was dependent on a constant supply of inoculum from an outside source. Well isolated commercial plantings were free of mildew.

Studies on Ceratostomella fimbriata from Sweet Potato and Sycamore. FEAZELL, G. D., AND W. J. MARTIN. A self-sterile strain of the sycamore canker stain fungus, nonpathogenic to sweet potatoes, was mated with a self-sterile strain of *C. fimbriata* from sweet potato. From the resulting line of perithecia 255 single ascospores were isolated and only three cultures, one self-sterile and two self-fertile, were established. The self-sterile culture was only slighty pathogenic to sweet potato, but the two self-fertile cultures, although definitely less pathogenic than the parent sweet potato culture, caused typical black-rot symptoms. On potato-dextrose agar these three single-ascospore cultures produced numerous mutants in the form of sectors and patches. The cultures obtained from sectors varied in pathogenicity to sweet potato, and certain of the patch mutants were nonpathogenic. Such frequent mutations had not been recorded previously in single-ascospore cultures of *C. fimbriata.* From one of the self-fertile cultures, 203 single-ascospore isolations gave rise to only 13 cultures. These were all self-fertile and had varying degrees of pathogenicity to sweet potatoes. It appears that hybridization occurred between these two morphologically similar, but genetically distinct, fungi from sweet potato and sycamore.

A Possible Adaptation of the Phytograph for Use in Interpreting Plant-Disease Research. GARREN, KENNETH H. The phytograph, a circle with zero at the center and factors measured along the radii, has come into considerable use in presenting ecological data. Most phytographs are based on four factors: abundance, frequency, perpetuation, and size. Points located on radii are connected to give a polygon, and multiple-factor

data from several species can be compared on the basis of size, shape, and skew of the resultant polygons. As shown by preliminary data from a dieback disease of sweetgum, the phytograph may be changed to a pathograph by use of disease factors of abundance, frequency, vigor, and amount. For pathographs, data would be taken from the same species, and graphs drawn for sites, or treatments, or perhaps varieties. Data for pathographs are obtained easily from diseases of perennials, but it should not be too difficult to get similar data from annuals. A pathograph should give a somewhat clearer picture of multiple-factor variance in disease than line or bar graphs. They should precede detailed statistical analysis. Pathographs should have a secondary usefulness in preventing detailed analysis of data not worthy of such.

Weed Control by Chemicals in a Nursery of Cypress. GOOCH, F. S. Weeds were controlled by chemicals and little hand weeding in a nursery of cypress, *Taxodium distichum*, in 1949. The chemicals used were the herbicidal oils Varsol, LHH1, and LHH6, and 60 per cent active Sodium trichloroacetic acid. LHH1, LHH6, and Varsol (which are hydrocarbon naphthas) were applied at rates ranging from 40 to 80 gal. per acre. The T. C. A. was applied diluted with water at the rate of 20, 40, and 60 lb. per acre. The seedlings were severely injured or killed with LHH6 and T. C. A., but they were tolerant to the dosage necessary for weed control with Varsol and LHH1. Varsol applied at 40, 60, and 80 gal. per acre severely injured 2.2, 4.2, and 5.5 per cent, respectively, of the seedlings; and at 40, 60, and 80 gal. per acre, gave 84.5, 90.2, and 89.7 per cent control of the weeds. LHH1 applied at 40, 60, and 80 gal. per acre severely injured 3.6, 4.5, and 15.8 per cent of the seedlings; and at 40, 60, and 80 gal. per acre, gave 83.7, 89.6, and 95.6 per cent control of the weeds. Considerable savings are indicated in weed control in cypress nurseries.

Treatment of Stratified Loblolly Pine and Shortleaf Pine Seed with Fungicidal Dusts. HAMILTON, J. R., AND L. W. R. JACKSON.

Inheritance of Resistance to Verticillium Wilt. HARRISON, GEORGE.

Control of a Root Rot of Pine Seedlings by Soil Fumigation. HENRY, BERCH W. Studies on the control of a root rot of longleaf, slash, and loblolly pine seedlings at the W. W. Ashe Nursery, Brooklyn, Mississippi, were initiated in 1947 by the Bureau of Plant Industry, Soils, and Agricultural Engineering in cooperation with Region 8 and the Southern Forest Experiment Station, U. S. Forest Service. The disease, of unknown cause, is evidenced on 1-year-old stock by symptoms that range from small, red, rough areas on the taproot, to blackening and roughening of the entire surface of the tap root with loss of most of the lateral roots, to rotting away of the lower taproot with a proliferation of laterals from the upper portion. The disease decreases stand in the seedbeds, increases cull, and decreases survival in field plantings. In presowing treatments of seedbeds, excellent control was obtained with ethylene dibromide (32 gal./acre of 20 per cent by volume solution) and methyl bromide gas (1 lb./100 sq. ft.). Chloropicrin (41 gal./acre) was slightly less effective. Dichlorobutene and allyl bromide gave fair control but were markedly inferior to the above. Dichloropropane-dichloropropene mixture was ineffective. Of chemicals tested other than fumigants, formaldehyde and allyl alcohol applied as soil drenches gave slight control, and several known fungicides incorporated in the seedbed were ineffective.

Breeding Red Clover for Resistance to Southern Anthracnose. HENSON, LAWRENCE.

Pod- and Seed-Spotting of Soybeans Caused by Helminthosporium vignicola. HOLDEMAN, Q. L.

Pea and Vetch Diseases in Louisiana. HORN, N. L., AND J. G. ATKINS, JR. A disease survey of pea and vetch plantings, carried out during the winter of 1948–1949, revealed that the Ascochytas were primarily associated with pea diseases and that the Colletotrichums were the most significant fungi involved in the vetch diseases. Inoculation tests with Creole and Austrian Winter peas showed that *Mycosphaerella pinodes* and *Ascochyta pinodella* produce a foot rot, whereas *A. pisi* does not. *Colletotrichum pisi* was often found associated with the Ascochyta fungi but was of much less importance. *Colletotrichum* sp. isolated repeatedly from vetch plants at Baton Rouge, Louisiana, caused extensive damage to common vetch (*Vicia sativa*). The fungus was designated as isolate 192. Although this isolate resembles *C. villosum* in some respects, it differs in certain cultural characteristics and pathogenicity. Isolate 192 can withstand summer temperatures (above 32° C.) in culture and grows well on acid media. *Vicia villosa* and *V. atropurpurea* are fairly resistant to the fungus, whereas *V. sativa*, *V.*

monanthos, and *V. pannonica* are susceptible. *Kabatiella nigricans* was also isolated from vetch plants in the field but appeared to be of less importance than isolate 192. *V. villosa* and *V. sativa* were susceptible to *K. nigricans* when inoculations were made in the greenhouse.

Some Aspects of Breeding Tobacco for Disease Resistance. JENKINS, WILBERT A. A number of flue-cured tobacco lines have been bred possessing excellent quality, average to better than average yielding capacity, and high to moderate resistance to such soil-borne diseases as black shank and Granville wilt. In these lines, high resistance is correlated with short, and moderate to low resistance with longer, vegetative cycle. Seedlings (as transplanted) are relatively susceptible in all lines, the resistance principle being developed in conjunction with vegetative or growth function. Root-mutilating agencies suppress this function, thus preventing the full expression of genetic resistance. All other factors being equal, plant type, quality, yield, and certain contrary aspects of resistance may be improved by increasing the length of the vegetative cycle. In order to accomplish this, several apparent linkages must be broken or new parental materials must be found which embody higher seedling resistance factors genetically compatible with those now in hand.

Blue Lupine Seed-Treatment Tests. LYLE, JAMES A. Although soil-borne diseases are responsible for some of the losses in fields of blue lupine, *Lupinus angustifolius* L., seed-borne diseases, such as anthracnose, cause considerable damage under certain conditions. A number of chemical seed treatments were evaluated to determine their effectiveness in controlling seed-borne diseases of lupine. Seed used in these tests was the regular commercial strain. All seed lots used germinated 80 per cent or better under standard germination tests. Seed lots showed from 5 to 15 per cent infection by anthracnose in a 5-year period. Replicated plantings were made at five locations in Alabama. The average emergence for five seasons from seed treated with Arasan (75 per cent tetramethyl thiuramdisulphide), Phygon (2,3,-dichloro-1,4-naphthoquinone), or Spergon (tetrachloro-p-benzoquinone) was 5 per cent greater than from untreated seed. Similar increases were obtained with some other materials for one to three seasons. Number and size of nodules were unaffected by any chemical seed treatment. Plantings were made in soil that had grown lupine previously, in soil in which lupine had never been grown, and in steam-sterilized soil. There was no correlation between seed treatment and time of planting.

The Segregation of Root-Knot Nematodes through Host-Resistance Screening Trials. MACHMER, J. H.

New Gladiolus Diseases. MAGIE, R. O.

Internal Cork of Sweet Potatoes in Louisiana. MARTIN, W. J. Over a period of three years at Baton Rouge, Louisiana, only one of the 10 lots of sweet potatoes which originated in 1947 from plants with ring-spotting of leaves produced crops of cork-affected potatoes. However, lots of sweet potatoes originating from roots with definite cork symptoms consistently produced crops of cork-affected potatoes. Mother potatoes core-grafted with cork-affected cores (introduced with a No. 3 cork-borer) gave rise to shoots that produced cork-affected potatoes, whereas the check mother potatoes gave rise to shoots that produced cork-free potatoes. Ring-spotting of leaves was equally prevalent in all plots during the three growing seasons. These results suggest the possibility that two causative agents might be involved in the production of ring spots on leaves and corky spots in roots of sweet potatoes. Heat treatments of mother potatoes and vine cuttings were not successful in destroying the agent or agents responsible for these symptoms.

Studies of Fusarium Wilt Resistance in Cotton, and Fiber Properties of Some Selected Strains and Hybrids. NEAL, D. C. Fusarium wilt resistance of 24 lines of cotton comprising selections and hybrids were studied in Louisiana from 1945 to 1949 in greenhouse and field. The incidence of wilt in the field during these years varied widely because of differences in rainfall during the growing seasons; only in 1945, 1947, and 1948 were weather conditions satisfactory for determining resistance. In the greenhouse a fairly satisfactory wilt differential was obtained by growing the plants in beds containing a mixture of artificially wilt-infested sand and silt loam and by inoculation at time of flowering and boll formation with agar cultures of the fungus. Among the lines studied, Coker 100 Georgia 97–2–2–2, Deltapine 6, Deltapine 6 × Delfos 6102, Deltapine 6 × Delfos 6102 × Deltapine 6, and Louisiana 33 were resistant in both greenhouse and field tests. Two lines were resistant in the greenhouse in 1949 but moderately susceptible in field tests in 1947. Improvement in qualities of fiber of some of these re-

sistant lines, such as lint percentage, staple length, uniformity, and tensile strength, has been obtained through selection.

Crinkle Leaf of Cotton in Louisiana and Arkansas in 1949. NEAL, D. C., W. J. PEEVY, AND V. H. YOUNG. Crinkle leaf of cotton, first reported in Louisiana in 1937, developed again in 1949, and during the years intervening there has been some increase in the prevalence of this physiological disease. In Louisiana it has occurred in East Baton Rouge, East Feliciana, West Feliciana, Livingston, and St. Landry parishes, being confined largely to the Mississippi River terraces and loessial-hill soils of the State particularly in the Lintonia and Olivier silt loams. The disease also developed in Arkansas in 1949, being observed in Arkansas, Chicot, Lawrence, Lincoln, Monroe, White, and Woodruff counties, in the soil types Calhoun, Caddo, Crowley, Lintonia, Olivier, Portland, Susquehanna, and other closely related silt loams and clays of the northeastern and southern Arkansas terraces. Crinkle leaf when first discovered in Louisiana was found to be associated with high soil acidity (pH range 4.5 to 4.9), calcium deficiency, and manganese toxicity. Determinations of the reaction of soil samples from the localities reporting the presence of the disease in Arkansas gave practically the same pH range. In areas where crinkle has been found in Louisiana, liming during the winter months has corrected it. Symptoms of the disease are puckered, mottled, partially chlorotic, and variously distorted leaves; fasciation of branches and shortened internodes; chlorophyll deficiency and reduction in size of involucral bracts, floral buds, flowers, and bolls.

The Essential Nature of Trace Elements in the Fertilizer Program. NIKITIN, A. A. Symptoms of trace element deficiencies in plants may be expressed by changes in size or color of fruit or foliage, by necrotic spots on fruits and other succulent tissue, dying back of twigs, or by changes in shape of root tip. The optimum soil concentration of individual trace elements varies for different crops and may be raised or lowered by the presence of other synergistic or antagonistic trace elements. The availability of trace elements is affected by the acidity of the soil. Most of the elements are more readily available when the pH of the soil is near 6.0. Some deficiencies which can be corrected, even after visible symptoms are noted, are: manganese deficiency of soybeans and peanuts, boron deficiency of celery and cauliflower, copper deficiency of citrus and tung, and zinc deficiency of corn and citrus. Copper, zinc, and manganese deficiencies of most crops can be corrected by foliage treatment with sprays or dusts. Neutral salts of the elements are most suitable. Foliage treatment permits a rapid evaluation of plant responses to trace elements and is an economical means of application.

Studies on Helminthosporium maydis and Helminthosporium carbonum Leaf-Spot Diseases of Corn. PATE, JAMES B. In 1947 a number of inbred lines, single and double crosses, and open-pollinated varieties of corn were artificially inoculated in the field with *H. maydis*. The infection was extremely heavy, and inbred lines varied from very resistant to very susceptible. The single crosses tested were generally resistant and the double crosses and open-pollinated varieties varied from resistant to moderately susceptible. The results indicated that resistance to *H. maydis* is dominant and that more than one factor is involved. Since NC37, one of the parent lines of the double cross hybrid Dixie 17, is susceptible to *H. carbonum* race 1, a program was initiated in 1946 to breed resistance into this inbred line. NC37 was crossed with several resistant lines. Self fertilization was begun after one or two backcrosses to NC37. Segregating populations were artificially inoculated and counts of resistant and susceptible plants made. Observed ratios in 1946 and 1947 agreed with the hypothesis that reaction is governed by a single gene pair, with resistance dominant. However, in 1948 and 1949 significant deviations from the expected 3:1 ratio were obtained. A number of resistant NC37 type lines have been recovered.

Susceptibility of Small Grains to Diseases in the South. PRESLEY, JOHN T.

Studies of Internal Cork of Sweet Potatoes. RANKIN, H. W. Internal cork of sweet potatoes is a virus disease. Insect transmission experiments show that leafhoppers probably are not vectors but that aphids (*Myzus persicae*) and perhap other species are. Use of steamed plots in combination with unsteamed plots and walk-in cages indicated soil-inhabiting insects are not vectors. Several insecticides used in field spray tests did not control the transmission of internal cork virus satisfactorily. Plants grown from potatoes with internal cork symptoms produced varying amounts of corky potatoes in field tests. In none of the plants were all the roots corky, and 16 per cent of the plants had no roots with internal cork. The expression of the internal cork symptoms appears to be influenced by environment. The Puerto Rico variety of sweet potato

is highly susceptible to internal cork (40 per cent with symptoms in 1949). Seventy-two other sweet potato varieties, selections, and seedlings, all grown in the field from 1 to 8 years and fully exposed to natural transmission, were examined for internal cork symptoms. Seventy-five per cent showed no symptoms. There is evidently considerable resistance within the species. No consistent above-ground symptoms were observed in sweet potatoes at Tifton, Georgia.

Variability of Cultures of Ascochyta gossypii and Its Pathogenicity on Cotton Seedlings. THOMPSON, GEORGE E. A total of 575 single conidia were isolated from single pycnidia in different leaf lesions and grown on potato-dextrose agar. The results showed that the fungus was composed of many different strains which could be distinguished by the color and amount of aerial mycelium, rate of growth, and production or nonproduction of pycnidia with conidia. Selected strains were grown on eight different media and it was found that the sporulating types usually produced conidia on all media, although the abundance varied. The nonsporulating strains produced a few pycnidia and conidia when grown on a medium composed of 100 gm. of dried ground cotton stems, 10 gm. agar, and 1000 ml. distilled water. The optimum temperature for growth in culture was approximately 20° C. for certain strains and 25° C. for others. Sterilized soil was inoculated with suspensions of conidia at the time of planting the seed. Infections developed on the base of the stems and on roots of the seedlings. Pycnidia with conidia were found in the lesions and the fungus was reisolated. Infected leaves, collected in the fall and wintered out-of-doors in wire baskets, contained pycnidia with viable conidia in June of the following year.

Soil Treatments against the Mimosa Wilt Fusarium. TOOLE, E. RICHARD. Although clones of the mimosa tree (*Albizzia julibrissin*) have been found which are highly resistant to the wilt caused by *Fusarium oxysporum f. perniciosum*, there is need for a practical method of keeping healthy, individual susceptible trees now growing in the wilt area. During 1949 a start was made on this problem by testing a number of chemicals in the laboratory and greenhouse. Ferbam (ferric dimethyl dithiocarbamate), Phygon (2,3-dichloro-1,4-naphthoquinone), Puratized (para amino phenyl cadmium dilactate), and oxyquinoline benzoate, four materials that appeared promising in laboratory tests, were applied to soil in flats in the greenhouse at rates of 100 lb. an acre. This soil was infested with the wilt Fusarium, and susceptible mimosa seedlings were planted in it at the time of treatment. After treatment the soil in each flat was sampled for abundance of Fusarium. Forty-four days after treatment, reduction in Fusarium was 100 per cent for ferbam, 85 per cent for Phygon, 55 per cent for Puratized, and 25 per cent for oxyquinoline benzoate. The number of trees wilted following each treatment were none for ferbam, 33 per cent for Phygon, 25 per cent for Puratized, 33 per cent for oxyquinoline benzoate, and 67 per cent for checks. Similar results were obtained in other greenhouse tests.

The Influence of Mosaic on Yield of Staked Tomatoes. WALTER, JAMES M. A factorial split-plot field study of tomato mosaic involved: (1) three types of viruses, namely tobacco mosaic, tobacco etch, and cucumber mosaic; (2) early, late, and no inoculation; (3) three chemical sprays, namely malachite green at 1:4000, zinc sulfate at 1:800, and Copper A at 1:200; and (4) two wilt-resistant tomato lines, W185-6 and MStW 210-5, the latter resistant to tobacco mosaic. All plots were sprayed weekly with Parathion insecticide, but stray cases of mosaic appeared in midseason and at the end of the season there were 8.71 per cent rogues from noninoculated plots. Roguing was ineffective because of the impossibility of early recognition of infected plants. All symptomatic fruits were graded as culls in the differentiation between marketable and total yields. Malachite green applied as a weekly spray influenced neither total nor marketable yield. Zinc sulfate significantly reduced total yield of W185-6 without affecting that of MStW 210-5. Neither tomato line showed response in marketable yields to zinc sulfate. Time of inoculation was not important to marketable yield of MStW 210-5, but on W185-6 early inoculation reduced marketable yield to approximately half that for late inoculation, which was less than half that for controls. The three viroses were equally harmful on W185-6. Etch and cucumber mosaic were equally damaging on MStW 210-5, which was not affected by tobacco mosaic.

Cercospora Leaf Spot, A Severe Disease of Tall Fescue and Smooth Brome. WHITEHEAD, MARVIN D. A leaf spot, *Cercospora festucae*, occurred in severe proportions on tall fescue and smooth brome at College Station and Temple, Texas. In a spaced planting of mature clones of alta fescue 100 per cent were severely infected and 36 per cent dead or with less than 10 tillers remaining alive. No mature clones of brome were noted as killed by the disease, although 50 per cent were severely infected. Leaf spots

first noted in April continued to develop and became more severe as summer progressed. The disease began to subside with occurrence of fall rains during October, although infections continued to appear on the vigorous new leaves. The organism attacks the foliage of tall fescue causing oval to elongate leaf spots 0.5–8 mm. in length with gray centers and purple-brown borders. Initial infections occur on the leaf tips; in spreading down the leaf the spots coalesce, inciting tip necrosis. On the foliage of smooth brome the spots are more elongate and commonly smaller and more numerous than on tall fescue; early tip necrosis imparts a general gray-black color to the brome. Drawings of the fungus and an amended description are presented to record the morphology of the fungus as it appears on these two economically important grasses.